**ROBUST SUMMARY**
**OF INFORMATION ON**

**Substance:** # CRUDE OIL

**CAS No**. # 8002-05-9

**Summary prepared by:** American Petroleum Institute

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

### 1.1.1 GENERAL SUBSTANCE INFORMATION

**Substance type** : Petroleum product
**Physical status** : Liquid

**Remark** : The CAS definition for Petroleum (Crude oil) is:

> "A complex combination of hydrocarbons. It consists predominantly of aliphatic, alicyclic and aromatic hydrocarbons. It may also contain small amounts of nitrogen, oxygen and sulfur compounds. This category encompasses light, medium, and heavy petroleums, as well as the oils extracted from tar sands".
>
> Hydrocarbonaceous materials requiring major chemical changes for their recovery or conversion to petroleum refinery feedstocks such as crude shale oils, upgrade shale oils and liquid coal fuels are not included in this definition.

Crude oil contains hydrocarbons in the carbon number range from C1 to C60+. It also contains organometallic complexes, notably of sulfur and vanadium, and dissolved gases such as hydrogen sulfide. Crude oils range from thin, light colored oils consisting mainly of gasoline-quality stock to heavy, thick tar-like materials.
An "average crude oil has the following general composition:

| | |
|---|---|
| Carbon | 84% |
| Hydrogen | 14% |
| Sulfur | 1-3% |
| Nitrogen | 1% |
| Oxygen | 1% |
| Minerals and salts | 0.1% |

The chemical composition of crude oils can vary tremendously from different producing regions and even from within a particular formation.

Examples of compositions of various whole crudes are shown in the following table

| **Crude source** | **Paraffins % vol.** | **Naphthenes % vol** | **Aromatics % vol** | **Sulfur % wt** | **API gravity (°API)** |
|---|---|---|---|---|---|
| Light crudes | | | | | |
| Saudi light | 63 | 18 | 19 | 2.0 | 34 |
| South Louisiana | 79 | 45 | 19 | 0 | 35 |
| Nigerian light | 37 | 54 | 9 | 0.1 | 36 |
| North sea Brent | 50 | 34 | 16 | 0.4 | 37 |
| Beryl | | | | | |

| | | | | |
|---|---|---|---|---|
| 47 | 34 | 19 | 0.4 | 37 |
| Lost Hills | | | | |
| Non-Aromatics 50% | | 50 | 0.9 | |

| | | | | |
|---|---|---|---|---|
| Heavy crudes | | | | |
| Prudhoe Bay | | | | |
| 27 | 36 | 28 | 0.9 | 28 |
| Saudi Heavy | | | | |
| 60 | 20 | 15 | 2.1 | 28 |
| Venezuela Heavy | | | | |
| 35 | 53 | 12 | 2.3 | 24 |
| Belridge Heavy | | | | |
| Non-aromatics 37% | | 63 | 1.1 | - |

| | | | | |
|---|---|---|---|---|
| Mid-range crudes | | | | |
| Kuwait | | | | |
| 63 | 20 | 24 | 2.4 | 31 |
| Venezuela Light | | | | |
| 52 | 34 | 14 | 1.5 | 30 |
| USA West Texas Sour | | | | |
| 46 | 32 | 22 | 1.9 | 32 |

Crude oils may be categorized in either of several different ways e.g.

Paraffinic vs naphthenic.
Crude oils contain both paraffinic and naphthenic hydrocarbons but if there is a preponderance of paraffinic hydrocarbons present, the crude oil is referred to as a paraffinic crude. These crudes would be rich in straight and branched chain paraffins. Conversely a crude in which naphthenic hydrocarbons are predominant is referred to as a naphthenic crude. These crudes contain mainly naphthenic and aromatic hydrocarbons

Sweet vs sour
Crude oils may be referred to as either sweet or sour depending on the level of hydrogen sulfide present. A sweet crude has very little $H_2S$ whereas a sour crude has larger quantities of $H_2S$ present.

Light vs heavy
Crude oils may be divided into Light and Heavy crudes on the basis of their gravity.
The API gravity is determined as:

$$°API = \frac{141.5}{\text{Specific gravity}} - 131.5$$

Crude oils with gravity > 33°API are considered as light crudes. Such crudes with a high percentage composition of hydrogen are usually more suitable for processing for gasoline production. Heavy crudes, ie those with gravity < 28°API tend to contain more asphaltenes and are usually rich in aromatics. These heavy crudes require more steps in their processing.

Information in this robust summary is presented for light and heavy crudes since this categorization distinguishes between crudes with a high paraffinic content (Light crudes) and those with a high aromatic/naphthenic content (Heavy crudes). This represents the extremes of the ranges of crudes available.

## 1.13 REVIEWS

**Memo** : IARC Review

**Remark** : IARC reviewed the evidence for carcinogenicity of crude oil to man and animals and published the result in 1989.
IARC concluded that:

There is inadequate evidence for the carcinogenicity in humans of crude oil

There is limited evidence for the carcinogenicity in experimental animals of crude oil

The overall evaluation was

Crude oil is not classifiable as to its carcinogenicity to humans (Group 3)

(38)

### 2.1 MELTING POINT

| | | |
|---|---|---|
| **Value** | : | -30 - 30 °C |
| **Sublimation** | : | No |
| **Method** | : | ASTM D97 |
| **GLP** | : | no data |
| **Remark** | : | The figures quoted are a typical range for the drop point as measured by a standard oil industry procedure. For some low wax crudes, pour points below -30 °C are obtained. |
| **Reliability** | : | (1) valid without restriction |

(1) (16)

### 2.2 BOILING POINT

| | | |
|---|---|---|
| **Value** | : | Approximately -1°C to over 720°C (30°F – 1328°F) at 1013 hPa |
| **Decomposition** | : | Yes |
| **Method** | : | ASTM D7169 |
| **GLP** | : | no data |
| **Remark** | : | This approximate range for crude oil is based on the boiling point of n-butane for the lower end and an upper estimate based on ASTM D7169 – 05, “Standard Test Method for Boiling Point Distribution of Samples with Residues Such as Crude Oils and Atmospheric and Vacuum Residues by High Temperature Gas Chromatography”. In practice, atmospheric distillation of crude oil is not practiced above 275-300 °C, to avoid thermal decomposition of the residue. The residue is normally vacuum distilled in a subsequent operation. |
| **Reliability** | : | (1) valid without restriction |

(1) (77)

### 2.4 VAPOUR PRESSURE

| | | |
|---|---|---|
| **GLP** | : | no data |
| **Test substance** | : | Crude oil |
| **Remark** | : | Vapor pressure measurements were provided for 10 petroleum crude oils originating from various locations throughout the world. The source of these data are from Environment Canada Environmental Technology Center, a government-maintained database (Jokuty et al., 2002). Data cited by OGJ may be considered a secondary source, but the data cited by ESD and EETD were measured data sponsored by those agencies using a standardized method for measuring vapor pressure of petroleum products[1]. As such, these 10 measurements provide a body of data adequate for use in the U.S. EPA HPV program and specific for the physical/chemical endpoint of vapor pressure.<br><br>(1) ASTM D323, Standard Test Method for vapor Pressure of Petroleum Products (Reid Method) |
| **Result** | : | Vapor Pressure, kPa See the following table and Remarks section<br>Temperature °C Not stated<br>Decomposition Not stated |

Vapor Pressures, kPa:

| Product Name | Origin | Reid Vapor Pressure Value, kPa | Reference |
|---|---|---|---|
| Alaska North Slope | Alaska USA | 19 | ESD 91 |
| Arabian Medium | Saudi Arabia | 22.1 | OGJ 99 |
| Alif | Yemen | 45 | OGJ 99 |
| Amna | Libya | 27 | OGJ 99 |
| Ashtart | Tunisia | 13 | OGJ 99 |
| Atkinson | Beaufort Sea, Canada | 6 | ESD 91 |
| Alberta Sweet Mixed Blend | Alberta, Canada | 19 | EETD 84 |
| Abu Al Bu Khoush | United Arab Emirates | 24 | OGJ 99 |
| Beryl | North Sea, UK | 36 | OGJ 99 |
| Bombay High | India | 33 | OGJ 99 |

**Reliability** : (2) valid with restrictions
Data obtained from a government (Environment Canada) database who sponsored the vapor pressure measurements cited by ESD and EETD.
(20) (21) (39) (74)

### 2.5 PARTITION COEFFICIENT

**Log pow** : 2 - 6
**Method** : Calculated

**Remark** : The calculation was done by the CLOGP Version 3.5 program (calculation of Log partition coefficient octanol/water). The figures represent the spread of calculated and/or measured values for typical hydrocarbon components of crude oil. Calculated values for higher molecular weight hydrocarbons will be above 6, but such values are notional, since no correlation has been established between calculated and experimental values.
**Reliability** : (1) valid without restriction
(16) (45)

### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

**Solubility in** : Water
**Method** : Preparation of water soluble fraction
**Year** : 1990
**GLP** : No
**Test substance** : Crude oil

**Method** : Individual saturated crude oil solutions were prepared by adding approximately 10 ml of the respective oil to 50-100 ml of water in 125 ml separatory funnels. Funnels were gently shaken for at least 48 hrs either with a magnetic stir bar or with a wrist action shaker, then placed in a temperature bath for at least 48 hr prior to analysis. Solubility at 5, 20 and 22 (±2C) °C was determined in both double distilled water and salt water (3% NaCl). The effect of water to oil ratio on the solubility of crude oil components was determined by injecting oil into sealed vials completely filled with water, and mixed at low speed for an equilibration period of 20

days. Analysis was determined by purge-and-trap/GC; solid sorbent extraction with HPLC and fluorescence analysis.

**Result** : Fluorescence/HPLC analysis was inadequate in quantification of hydrocarbon composition.
Purge and trap/GC analysis results based on total benzene, toluene, ethyl benzene+xylenes (combined concentration) and naphthalenes (BTE+XN as mg/l) are reported along with viscosity (vis=c.p @ 20°C) and density (d=g/cm$^3$ @ 20°C) for each oil at respective temperatures (°C) in distilled and saltwater (22°C reported as ± 2°C)

| | BTE+XN (mg/l) | |
|---|---|---|
| **Oil** | **Distilled water** | **Saltwater** |
| Norman Wells<br>4.41 vis; 0.832 d | | |
| | 30 (5°C)<br>29-33 (20°C) | 25.5 (5°C) |
| | 31.8-33.5 (22°C) | 20 (22°C) |
| 4.7 vis; 0.84 d | 33 (20°C) | 14.8 (5°C) |
| Alberta: 4.98 vis; 0.835 d | 25.02(22°C) | |
| Swan Hills: (no vis. density) | 35.1 (22°C) | |
| Prudhoe Bay: 34.9 vis; 0.901 d | 29.01 (22°C) | |
| Lago Medio: 41.1 vis; 0.872 d | 23.66-25.5 (22°C) | 16.47 (22°C) |
| Kopanoar: 17.5 vis; 0.9 d | 10.42 (22°C) | |
| Murban: 4.37 vis; 0.824 d | 28.62 (22°C) | |
| Hibernia B 33.2 vis; 0.837d | | 16.92 (22°C) |
| Hibernia J (no vis., density) | | 7.75 (22°C) |
| Mobil A (no vis., density) | 29.6 (22°C) | |
| Mobil B (no vis., density) | 58 (22°C) | |

Results for oil-water ratio testing were not quantified, but general observations were stated as follows: concentrations of the water soluble fractions decreases as the water-to oil ratio increases and the composition of the water soluble fraction changes as the ratio changes. At low water to oil ratios, the WSF is composed predominantly (80%) of BTEX. As the water to oil ratio increases, these compounds account for a smaller proportion of the dissolved compounds. At a water/oil ratio of 10000, these compounds account for only 15-30% of the total WSF.

**Conclusion** : Limited detail is provided for exact amounts of crude oil used for preparing aqueous solutions, nor is there any information regarding the composition of each of the crude oils tested, either as hydrocarbon type or inorganic components (such as sulfur). Also, no information on the GC calibration standard composition used to identify and quantify soluble components in the equilibrated aqueous -oil solutions is provided. Individual components of complex petroleum substances have specific and differing solubilities. At any particular loading rate, the resulting aqueous concentration of each chemical constituent is a function of the relative volume of the two phases (aqueous and the petroleum mixture), the partition coefficient between the phases, the amount of component present and the maximum water solubility of each component. Initially as the petroleum mixture is added in amounts below the solubility limit of the least soluble component the aqueous concentration increases proportionally until the least soluble

component reaches a saturation concentration, and only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the petroleum mixture results in an aqueous concentration that is a non-linear function of the amount added.

**Reliability** : (2) valid with restrictions

(14) (15) (18) (19) (47) (75)

## 2.14 ADDITIONAL REMARKS

**Memo** : CONCAWE

**Remark** : Petroleum is a natural organic material consisting mainly of hydrocarbons. It occurs in both the gaseous and liquid states in geological traps. The liquid phase, after being freed from dissolved gas and any associated salt water, is known as crude oil. All the information presented in this Data Set relates to crude oil.
Crude oil is not a uniform substance since its physical and chemical properties vary from oilfield to oilfield and can even vary within wells at the same oilfield. At one extreme, crude oil is a light, mobile, straw-colored liquid containing a large proportion of hydrocarbons which are readily distilled at atmospheric pressure. At the other extreme, crude oil is a highly viscous, semi-solid, black substance from which little can be distilled at atmospheric pressure before thermal decomposition occurs.
The most consistent property of crude oils is their relatively small range of elemental composition, as the ranges in the following table show:

| element | composition (wt%) |
|---|---|
| carbon | 83.9 - 86.8 |
| hydrogen | 11.0 - 14.0 |
| sulfur | 0.06 - 8.0 |
| nitrogen | 0.02 - 1.7 |
| oxygen | 0.08 - 1.82 |
| metals | 0.00 - 0.14 |

Crude oils are normally characterized in terms of three properties; density, viscosity and sulfur content. Crude oils are identified as either light (specific gravity <0.82), or medium (specific gravity 0.82 to 0.97), or heavy (specific gravity > 0.97). The viscosity is an expression of the mobility of the crude oil. The sulfur content has a marked influence on the refinery procedures to which the crude oil, and in particular its derivatives, will be subjected in order to produce acceptable products.
Crude oils are also characterized in terms of their chemical composition, specifically on the predominance of the hydrocarbon types that are present. Modern practice tends to recognize two main types of crude, namely paraffinic and naphthenic. Paraffinic crude oils are rich in straight-chain and branched-chain alkanes, whereas in naphthenic crudes the main constituents are cycloparaffins and aromatic hydrocarbons. However, this is a simplified picture, as many crude oils fall between or outside these two types.

**Source** : The technical information has been compiled by the Oil Companies' European Organization for Environmental Health Protection (CONCAWE), based at Madouplein-1, B-1210 Brussel, Belgium, and this organization holds copies of the reference articles cited in this data set

**Reliability** : (2) valid with restrictions

(5)

### 3.1.1 PHOTODEGRADATION

**Type** : air
**Light source** : Sun light
**INDIRECT PHOTOLYSIS**
**Sensitizer** : OH
**Conc. of sensitizer** : 1000000 molecule/cm³
**Method** : Calculated according to Atkinson, 1990

**Remark** : Atkinson gives data which enables half lives to be calculated for the degradation of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight. Values for typical hydrocarbon constituents of crude oils are as follows:

| **Constituent** | **Half-life (days)** |
|---|---|
| benzene | 6.5 |
| n-butane | 3.2 |
| n-hexane | 1.4 |
| toluene | 1.3 |
| cyclohexane | 1.1 |
| n-decane | 0.69 |
| n-tetradecane | 0.42 |
| naphthalene | 0.37 |

Hydrocarbons of carbon number greater than C20 will have little or no tendency to partition to air (see Sub-chapter3.2.2).

**Reliability** : (2) valid with restrictions

(3) (16)

### 3.1.2 STABILITY IN WATER

**GLP** : no

**Remark** : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters and sulfonic acid esters. The chemical components found in the materials that comprise the crude oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.

**Reliability** : (1) valid without restriction

(36)

### 3.3.2 DISTRIBUTION

| **FATE<br>TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS<br>FUGACITY/DISTRIBUTION** | |
|---|---|
| **Category Name:** | **CRUDE OIL** |
| **Category Chemical :** | Crude Oil |
| **Test Substance :** | Crude Oil, CAS RN 8002-05-9 |
| **Test Substance Purity/Composition and Other Test Substance Comments:** | |
| **Category Chemical Result Type :** | Estimated by calculation |
| **Test Substance Result Type :** | Estimated |
| **RESULTS** | |
| **Fugacity/Distribution Result Description:** | Crude oil is a substance of variable composition containing hydrocarbons with carbon numbers typically from one (methane) to complex molecules of 60 or more carbon atoms. Multimedia distribution was calculated for various alkyl and aryl hydrocarbons typically found in crude oil. This provided an understanding of the distribution spectrum that might be expected for these constituents. |
| **Test Results :** | |

| | **Percent Distribution** | | | | | |
|---|---|---|---|---|---|---|
| | **air** | **water** | **soil** | **sediment** | **susp. sediment** | **biota** |
| propane | 100 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| n-butane | 100 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| n-hexane | 100 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| n-octane | 100 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| n-decane | 100 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| n-tetradecane | 67 | <0.1 | 33 | 0.7 | <0.1 | <0.1 |
| n-eicosane | <0.1 | <0.1 | 98 | 2.0 | <0.1 | <0.1 |
| cyclohexane | 100 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| benzene | 99 | 0.9 | <0.1 | <0.1 | <0.1 | <0.1 |
| toluene | 99 | 0.8 | 0.4 | <0.1 | <0.1 | <0.1 |
| p-xylene | 98 | 0.6 | 0.9 | <0.1 | <0.1 | <0.1 |
| o-xylene | 98 | 0.8 | 0.9 | <0.1 | <0.1 | <0.1 |
| ethylbenzene | 98 | 0.6 | 0.8 | <0.1 | <0.1 | <0.1 |
| n-butylbenzene | 93 | 0.3 | 6.3 | 0.1 | <0.1 | <0.1 |

| | |
|---|---|
| **Temperature :** | 20°C |
| **Level of Multi-media** | 1 |

**Model :**

**Model Input (Water Solubility:)**

| | Water solubility (mg/L) |
|---|---|
| propane | 62.4* |
| n-butane | 64.2* |
| n-hexane | 9.5* |
| n-octane | 0.66* |
| n-decane | 0.052* |
| n-tetradecane | 0.0022* |
| n-eicosane | 0.0019* |
| cyclohexane | 55* |
| benzene | 1790* |
| toluene | 526* |
| p-xylene | 162* |
| o-xylene | 178* |
| ethylbenzene | 169* |
| n-butylbenzene | 11.8* |

Values denoted by * were cited in the EPI-Suite experimental database.

**Model Input (Vapor Pressure:)**

| | Vapor Pressure (Pa) |
|---|---|
| propane | 953000* |
| n-butane | 243000* |
| n-hexane | 20100* |
| n-octane | 1888* |
| n-decane | 191* |
| n-tetradecane | 1.55* |
| n-eicosane | 0.00062* |
| cyclohexane | 12900* |
| benzene | 12600* |
| toluene | 3790* |
| p-xylene | 1180* |
| o-xylene | 1060* |
| ethylbenzene | 1280* |
| n-butylbenzene | 141* |

Values denoted by * were cited in the EPI-Suite experimental database.

**Model Input (log $K_{ow}$: )**

| | Partition Coefficient (Log Kow) |
|---|---|
| propane | 2.4* |
| n-butane | 2.9* |
| n-hexane | 3.9* |
| n-octane | 5.2* |
| n-decane | 5.0* |
| n-tetradecane | 7.2* |
| n-eicosane | 10.2 |
| cyclohexane | 3.4* |
| benzene | 2.1* |
| toluene | 2.7* |
| p-xylene | 3.2* |
| o-xylene | 3.1* |
| ethylbenzene | 3.2* |
| n-butylbenzene | 4.4* |

Values denoted by * were cited in the EPI-Suite experimental database.

**Model Input ( Melting Point: )**

| | Melting Point (°C) |
|---|---|
| propane | -187.6* |
| n-butane | -138.2* |
| n-hexane | -95.3* |

| | |
|---|---|
| | n-octane -56.8*<br>n-decane -29.7*<br>n-tetradecane 5.8*<br>n-eicosane 36.8*<br>cyclohexane 6.6*<br>benzene 5.5*<br>toluene -94.9*<br>p-xylene 13.2*<br>o-xylene -25.2*<br>ethylbenzene -94.9*<br>n-butylbenzene -87.9*<br>Values denoted by * were cited in the EPI-Suite experimental database. |
| **Henry's Law Constant :** | Calculated by EQC for each constituent |
| **Model Concentration -- Air :** | |
| **Model Concentration -- Water :** | |
| **Model Concentration -- Soil :** | |
| **Model Concentration -- Sediment :** | |
| **Results Remarks :** | |
| **STUDY/METHOD** | |
| **Key Study Sponsor Indicator :** | Key |
| **Year Study Performed :** | |
| **Method/Guideline Followed :** | EQC Equilibrium Criterion Model, Fugacity Based Level 1 |
| **Deviations from Method/Guideline :** | |
| **Method/Guideline Description :** | The EQC model calculates the distribution of a fixed quantity of conserved (i.e., non-reacting) chemical, in a closed environment at equilibrium, with no degrading reactions, no advective processes, and no intermedia transport processes (e.g., no wet deposition or sedimentation). The medium receiving the emission is unimportant because the chemical is assumed to become instantaneously distributed. |
| **Method/Guideline and Test Condition Remarks :** | |
| **GLP :** | No |
| **Study Reference :** | Trent University. 2003. EQC fugacity-based EQC-equilibrium criterion model, Version 2.02. Canadian Environmental modeling Centre, Trent University, Ontario. URL: http://www.trentu.ca/cemc/ |
| **RELIABILITY/DATA QUALITY** | |

| | |
|---|---|
| **Reliability :** | (2) Reliable with restrictions |
| **Reliability Remarks :** | Environmental distribution was estimated using an accepted validated model. |

### 3.6 BOD5, COD OR BOD5/COD RATIO

**BOD5**

**Method** : Theoretical Oxygen Demand (TOD) was calculated
**Year** : 1971
**GLP** : no data

**Method** : Tests were run at 30 °C in fresh water using a respirometric procedure with crude oil at 50 and 70 mg/l. Prior to the test, the crude was "topped" at up to 100 °C to remove light ends, which comprised about 10% of the material

**Result** : The BOD/TOD ratio after 10 days was 0.3 at 50 mg/l and 0.04 at 70 mg/l. With the addition of nutrients, using ammonia at up to 4.6 mg/l of nitrogen and phosphate at up to 15 mg/l of phosphorus, the BOD/TOD ratio increased to a maximum of 0.3 after 5 days , and 0.34 after 10 days. In further studies, run at 10 °C in sea water in the presence of nutrients, the degradation rate was found to be lowered by a factor of 2 to 3 below that at 30 °C. The reference quotes the BOD5/TOD ratio, but does not give the individual values for BOD5 and TOD.

**Reliability** : (2) valid with restrictions

(7) (16)

### 3.8 ADDITIONAL REMARKS

**Memo** : General

**Remark** : The world production of crude oil per year is of the order of 3 billion tonnes per year, of which about half is transported by sea. In his 1989 publication, Clark states that the best estimate of petroleum hydrocarbons entering the sea per year is about 3 million tonnes, of which about one million tonnes is attributable to crude oil. Such pollution arises from the cleaning of oil tanker compartments, offshore oil production operations, discharge of coastal refinery effluent and spillage from oil tankers. Crude oil also enters the oceans by natural seepage from undersea locations. Particular attention has focused on major oil tanker spillages, notably involving the Torrey Canyon in 1969, the Amoco Cadiz in 1978 and the Exxon Valdez in 1990. As a result, the processes determining the fate of oil in seawater are reasonably well understood and have been reviewed by Atlas and Bartha. Initially, the oil spreads out as a film on the sea surface as a result of wind and wave action. The more volatile, lower molecular weight hydrocarbons are lost by evaporation. Polar compounds and the mono-aromatic hydrocarbons have an appreciable water solubility and are taken into solution. A key ancillary process is that of emulsification, since crude oil has a natural tendency to form emulsions in sea water. Such emulsions are usually of the oil-in-water type, but may also be of the water-in-oil type. The latter are often of the intractable 'chocolate mousse' type. Significant amounts of crude oil, particularly the higher molecular weight compounds, sink naturally, rolling along the ocean bottom picking up sand and shells and forming tarry balls which are resistant to degradation by any method. Hydrocarbons may also reach the bottom sediments by sorption onto suspended particles which ultimately settle on the sea floor. Spilt oil also

undergoes chemical changes, particularly oxidation by free radical mechanisms initiated by sunlight.
The initial products of such reactions are hydroperoxides, and these in turn form compounds such as alcohols, acids and aldehydes, many of which have an appreciable water solubility. Polymerization also occurs to yield intractable tarry materials.
The bulk of spilt crude oil is biodegraded by the micro-organisms present in sea water. Emulsification to form oil-in-water emulsions yields small particles of crude oil that are biodegraded by bacteria, yeasts, fungi and actinomycetes. Many factors influence the rate of biodegradation, in particular temperature, dissolved oxygen concentration and the availability of nitrogen and phosphorus nutrients. Adapted micro-organisms are often found in ocean areas where crude oil spills are common. Zobell has calculated that where an adapted microbial population is available in well-aerated sea water at 20 to 30 °C, the rate of crude oil oxidation ranges from 0.02 to 0.2 g of oil oxidized/$m^2$/day. The same author found experimentally that complete oxidation of 1.0 mg of hydrocarbon requires between 3 and 4 g of oxygen, i.e. it has a BOD of 3 to 4 mg oxygen/mg. Since the oxygen content of sea water is between 6 and 11 mg/liter, depending on salinity and temperature, this means that about 320 000 liters of sea water is required to oxidize one liter of crude oil. Crude oil contains hydrocarbons of well-defined generic types that are biodegraded at different rates. n-Alkanes are readily degraded in sea water, since many micro-organisms can utilize them. Branched-chain or iso-alkanes are less readily biodegraded but they do ultimately biodegrade. The degradation of cycloalkanes has not been extensively studied, but the ring structure is resistant to biodegradation. Aromatic hydrocarbons are also resistant to biodegradation, but a few micro-organisms are able to utilize them. High molecular weight compounds, the tars and asphaltenes, degrade very slowly.

(4) (12) (16) (17) (80) (81)

## 4.1 ACUTE/PROLONGED TOXICITY TO FISH

| Test Substance | |
|---|---|
| **Category Name: CRUDE OIL** | |
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Arabian medium crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The test oil was weathered, having a final volume 30 – 35% less than fresh oil volume. The weathered oil had the following characteristics:<br><br>Specific gravity .....0.9129<br>API gravity .....23.5<br>Reid vapor pressure.....2.1 KPa<br>Viscosity @ 15 .....102.4 CST<br>Viscosity @20 .....80.7CST<br>Pour point .....-14°C<br>Sulfur content.....2.96 weight % |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2001 |
| **Method/Guideline Followed**: | EPA (1985), Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, EPA/600/4-85/013. |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Menidia beryllina (inland silversides) |
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static-renewal; 24-h intervals |
| **Test Vessel**: | 500-mL amber bottles; sealed, no headspace |
| **Water Media Type**: | seawater, filtered and adjusted to a salinity of 20 parts per thousand |

| | |
|---|---|
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | Mean measured (Test 1): 0 (control), 1.58, 1.65, 3.03, 4.15, 5.18 mg TPH/L<br>Mean measured (Test 2): 0 (control), 0.83, 1.38, 2.93, 4.38, 4.79 mg TPH/L |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 5.0 – 7.4 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.1 Upper Range : 7.9 |
| **Test Temperature and Units**: | Value: 25°C<br>Lower Range :<br>Upper Range : |
| **Photo (Light/Dark)**: | 16 h light: 8 h dark |
| **Salinity**: | 20 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Exposure solutions consisted of independently-made water accommodated fractions (WAF) of the test oil. WAFs were prepared in either 2- or 4-L glass aspirator flasks. The sidearm of each flask was closed off with a short length of silicone tubing and a clamp. Crude oil was added by weight directly onto the pre-measured dilutions water with a gas tight syringe, giving loading in units of mg oil/L water. The flasks were sealed with Teflon stoppers, and placed on magnetic stir plates. The speeds were adjusted to zero vortex. WAF solutions were mixed for approximately 48 hours at room temperature (25±2°C). WAFs were immediately drawn from the bottom of the mixing flasks and placed in exposure chambers. Exposure chambers were 500-mL amber glass jars with Teflon-lined lids. Each jar was filled with respective WAF to within a few millimeters of the rim before organisms were added. Once organisms were added the chambers were sealed with the Teflon-lined lids to prevent loss of volatile components. Every 24 hours, 75% of the solution in each chamber was removed by straining through Nitex (100 micron) mesh to prevent loss of organisms and replaced with fresh test solutions and resealed.<br><br>Menidia were obtained from Charles Rivers, Inc., and were 7 days old. |

| | |
|---|---|
| | Organisms were acclimated for 3 days in a 40-L glass aquarium with salinity-adjusted seawater to 20 parts per thousand. The organisms were fed *Artemia* sp. nauplii *ad libitum*.<br><br>Definitive mortality observations were made after the 96-hour exposure. Mortality was based on the number of organisms added to the chamber minus the number of live organisms recovered. Dead organisms were those that showed no response to gentle prodding. LC50 values were calculated using ToxCalc 5.0 software package.<br><br>Samples of the fresh WAF solutions were taken at the beginning of each renewal period and analyzed for Total Petroleum Hydrocarbons (TPH) as defined by CROSERF as the resolved hydrocarbons ranging from C10 – C36 (Coelho and Aurand, 1997). This analysis included liquid-liquid extraction with methylene chloride. The gas chromatography-mass spectrometry (GC-MS) analysis was conducted on a HP5890 II GC coupled to a HP5972A MS. Toxicity results are expressed on daily mean TPH concentrations.<br><br>Two independent tests were run, with the endpoints provided in the following table. |
| **Limit Test**: | N/A |

### Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | 5.5 | | mg/L | death | Measured TPH |
| 96 | hours | LC | 50 | = | 4.9 | | mg/L | death | Measured TPH |

**Results Remarks**:

The following dose-response pattern was observed:

| Mean Conc mg/L | Number Surviving | Surviving/ Total | % Survival | |
|---|---|---|---|---|
| **TEST 1** | | | | |
| 0 (control) | 15 | 15/15 | 100 | |
| 1.58 | 12 | 12/15 | 80 | |
| 1.65 | 12 | 12/15 | 80 | LC50 = 5.5 mg/L |
| 3.03 | 10 | 10/15 | 67 | |
| 4.15 | 7 | 7/15 | 47 | |
| 5.18 | 9 | 9/15 | 60 | |
| **TEST 2** | | | | |
| 0 (control) | 16 | 16/16 | 100 | |
| 0.83 | 14 | 14/16 | 88 | |
| 1.37 | 10 | 10/15 | 67 | LC50 = 4.9 mg/L |
| 2.93 | 10 | 10/15 | 67 | |

| | | | | |
|---|---|---|---|---|
| | 4.38<br>4.79 | 5<br>11 | 5/15<br>11/17 | 33<br>65 |

| | |
|---|---|
| **Reliability/Data Quality** | |
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. |
| **Key Study Sponsor Indicator**: | |
| **Reference** | |
| **Reference**: | Fuller, C. and J. Bonner. 2005. Results of the Cooperative API/Texas Testing Program. Section 6 in: Aurand, D. and G. Coelho (eds.). Cooperative aquatic toxicity testing of dispersed oil and the "Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF)." Technical Report 07-03. Ecosystem Management & Associates, Inc. Lusby, Maryland. 105 pp. |

| | |
|---|---|
| **Test Substance** | |
| **Category Name: CRUDE OIL** | |
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Arabian medium crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The test oil was weathered, having a final volume 30 – 35% less than fresh oil volume. The weathered oil had the following characteristics:<br><br>Specific gravity ..... 0.9129<br>API gravity ..... 23.5<br>Reid vapor pressure ..... 2.1 KPa<br>Viscosity @ 15 ..... 102.4 CST<br>Viscosity @20 ..... 80.7CST<br>Pour point ..... -14°C<br>Sulfur content..... 2.96 weight % |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2001 |
| **Method/Guideline Followed**: | EPA (1985), Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, EPA/600/4-85/013. |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Cyprinodon variegatus (sheepshead minnow) |

| | |
|---|---|
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static-renewal; 24-h intervals |
| **Test Vessel**: | 500-mL amber bottles; sealed, no headspace |
| **Water Media Type**: | seawater, filtered and adjusted to a salinity of 20 parts per thousand |
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | Mean measured (Test 1): 0 (control), 3.12, 5.09, 4.72, 5.94, 6.73 mg TPH/L<br>Mean measured (Test 2): 0 (control), 1.99, 5.42 mg TPH/L |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 5.0 – 7.4 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.1 Upper Range : 7.9 |
| **Test Temperature and Units**: | Value: 25°C<br>Lower Range :<br>Upper Range : |
| **Photo (Light/Dark)**: | 16 h light: 8 h dark |
| **Salinity**: | 20 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Exposure solutions consisted of independently-made water accommodated fractions (WAF) of the test oil. WAFs were prepared in either 2- or 4-L glass aspirator flasks. The sidearm of each flask was closed off with a short length of |

| | |
|---|---|
| | silicone tubing and a clamp. Crude oil was added by weight directly onto the pre-measured dilutions water with a gas tight syringe, giving loading in units of mg oil/L water. The flasks were sealed with Teflon stoppers, and placed on magnetic stir plates. The speeds were adjusted to zero vortex. WAF solutions were mixed for approximately 48 hours at room temperature (25±2°C). WAFs were immediately drawn from the bottom of the mixing flasks and placed in exposure chambers. Exposure chambers were 500-mL amber glass jars with Teflon-lined lids. Each jar was filled with respective WAF to within a few millimeters of the rim before organisms were added. Once organisms were added the chambers were sealed with the Teflon-lined lids to prevent loss of volatile components. Every 24 hours, 75% of the solution in each chamber was removed by straining through Nitex (100 micron) mesh to prevent loss of organisms and replaced with fresh test solutions and resealed.<br><br>C. variegatus were purchased from Aquatic Biosystems, and were 3 days old. Organisms were acclimated overnight in salinity-adjusted seawater to 20 parts per thousand. The organisms were fed *Artemia* sp. nauplii *ad libitum*. They were used in testing at 4 days old.<br><br>Definitive mortality observations were made after the 96-hour exposure. Mortality was based on the number of organisms added to the chamber minus the number of live organisms recovered. Dead organisms were those that showed no response to gentle prodding. LC50 values were calculated using ToxCalc 5.0 software package.<br><br>Samples of the fresh WAF solutions were taken at the beginning of each renewal period and analyzed for Total Petroleum Hydrocarbons (TPH) as defined by CROSERF as the resolved hydrocarbons ranging from C10 – C36 (Coelho and Aurand, 1997). This analysis included liquid-liquid extraction with methylene chloride. The gas chromatography-mass spectrometry (GC-MS) analysis was conducted on a HP5890 II GC coupled to a HP5972A MS. Toxicity results are expressed on daily mean TPH concentrations.<br><br>Two independent tests were run, with the endpoints provided in the following table. |
| **Limit Test**: | N/A |

## Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | 4.2 | | mg/L | death | Measured TPH |
| 96 | hours | LC | 50 | = | 3.9 | | mg/L | death | Measured TPH |

| | |
|---|---|
| **Results Remarks**: | The following dose-response pattern was observed:<br>Mean<br>Conc Number Surviving/ % |

| | | | | | |
|---|---|---|---|---|---|
| | mg/L | Surviving | Total | Survival | |
| | **TEST 1** | | | | |
| | 0 (control) | 15 | 15/15 | 100 | |
| | 3.12 | 15 | 15/15 | 100 | |
| | 5.09 | 3 | 3/15 | 20 | LC50 = 4.2 mg/L |
| | 4.72 | 0 | 0/15 | 0 | |
| | 5.95 | 0 | 0/15 | 0 | |
| | 6.73 | 0 | 0/15 | 0 | |
| | **TEST 2** | | | | |
| | 0 (control) | 15 | 15/15 | 100 | |
| | 1.99 | 15 | 15/15 | 100 | |
| | 5.42 | 4 | 4/15 | 27 | LC50 = 3.9 mg/L |

| **Reliability/Data Quality** | |
|---|---|
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. |
| **Key Study Sponsor Indicator**: | |

| **Reference** | |
|---|---|
| **Reference**: | Fuller, C. and J. Bonner. 2005. Results of the Cooperative API/Texas Testing Program. Section 6 in: Aurand, D. and G. Coelho (eds.). Cooperative aquatic toxicity testing of dispersed oil and the "Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF)." Technical Report 07-03. Ecosystem Management & Associates, Inc. Lusby, Maryland. 105 pp. |

| **Test Substance** | |
|---|---|
| **Category Name: CRUDE OIL** | |
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Alaska North Slope crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The crude oil was reported to contain approximately 1/3 (w/w) volatile components; those hydrocarbons with a boiling point of 204°C to 274°C or less. |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |

| **Method** | |
|---|---|
| **Year Study Performed** : | 1998 |
| **Method/Guideline Followed**: | Generally followed ASTM E729 and OECD 203 |
| | |
| **Deviations from Method/Guideline** : | |

| | |
|---|---|
| **Species**: | Menidia beryllina (inland silversides) |
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static-renewal; 24-hour cycle |
| **Test Vessel**: | 400-mL beakers; covered with <20% headspace |
| **Water Media Type**: | Reconstituted saltwater using de-ionized water (≥18 MΩ-cm) and Crystal Sea Marinemix. |
| **Test Concentrations**: | Control and five WAF treatments of increasing loading rate. |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 5.8 – 8.3 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.44 Upper Range : 8.70 |
| **Test Temperature and Units**: | Value:<br><br>Lower Range : 22.0 °C<br>Upper Range : 28.5 °C |
| **Photo (Light/Dark)**: | 16 h light: 8 h dark<br>Light Intensity = 10 – 20 µE/$m^2$/s |
| **Salinity**: | 19.54 – 21.24 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline**<br>**Test Conditions Remarks**: | Water accommodated fractions of Alaska North Slope crude oil were independently created by adding a measured mass of oil to 3.5 L saltwater in a 4-L aspirator bottle. The WAF was created using a standardized method of low-energy mixing such that no vortex was created (0% water depth). Headspace in the aspirator bottle was approximately 25%, and all bottles were covered with aluminum foil. The temperature at which the WAFs were created was regulated at the testing temperature of 25°C. The WAFs were mixed for 24 hours, allowed to stand for 5 minutes and then collected for chemical analysis and immediate dispensing to the test vessels. Each WAF was taken from the bottle 90% of the water depth through the aspirator bottle's sampling port. Each control and WAF treatment was replicated three times.<br><br>Test vessels were 400-mL polypropylene beakers. The volume held in each beaker was not stated, but it was noted that the headspace was kept to <20%. Beakers were covered with a glass watchglass. Every 24 hours, 90% of the test solutions were decanted and fresh WAF was replaced. A low aeration rate (1.68 – 3.35 $cm^3$/min) was used to maintain dissolved oxygen.<br><br>The test animals used in the study were larval M. beryllina purchased from Aquatic Biosystems, Inc. (Ft. Collins, CO). They were acclimated for two days prior to test initiation. Test animals were 12 days old at test initiation. They were fed 1 mL of satwater-rinsed, concentrated, newly-hatched brine shrimp nauplii (Artemia, approximately 100 Artemia per animal) once or twice daily prior to and during the test.<br><br>WAF solutions were analyzed using gas chromatography/flame ionization detection (GC-FID) by US EPA SW-846 methods 5030, 8000B, and 8021B, and ADEC method AK101 and AK102. Solutions were measured for total volatile organic analytes (VOA; range defined as C6-C9) and total petroleum hydrocarbon content (TPH; range defined as C10-C36). The sum of the two fractions was calculated and termed the total hydrocarbon content (THC). Measured values of VOA were determined on a composite sample of WAFs collected from days 1 – 4. TPH was measured only from the initial WAF. This was adopted after verifying that the TPH concentration in each WAF was consistently low in relationship to VOA regardless of the loading rate. This was due to the limited solubility of the C10 – C36 hydrocarbons. |
| **Limit Test**: | N/A |

## Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | 15.59 | | mg/L | death | Measured THC |
| 96 | Hours | LL | 50 | = | 1641 | | mg/L | death | WAF loading rate |

| | |
|---|---|
| **Results Remarks**: | The test endpoint was presented based on the measured total hydrocarbon content (sum of VOA and TPH) and the WAF loading rate. Because aeration was applied to each test chamber, there was a |

| | |
|---|---|
| | potential for loss of the volatile fraction. This would underestimate the degree of toxicity because exposures were not constant over time. To assess the degree of underestimation of the toxicity values, an analysis of the change in VOA concentrations over time was made. A series of samples were collected and analyzed for VOA from the high and low WAF concentrations during the first 24 hours of a simulated exposure test. The VOA would be expected to be the fraction most affected by aeration. The measured concentrations were plotted against time and the area under the curve (AUC) was calculated. The AUC for the measured concentrations was compared against the AUC for a theoretical constant exposure. The VOA concentrations in the WAFs declined to near detection limits in approximately 12 hours, causing the AUC to be 90% less than the theoretical exposure. Although test solutions were renewed at 24-h intervals during the 96-hour test, these data indicate that the measured endpoints are likely to underestimate the toxicity that would be expected had constant exposures been maintained. |
| **Reliability/Data Quality** | |
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. Although solutions were renewed daily, the use of aeration likely caused some loss of volatile components. |
| **Key Study Sponsor Indicator**: | |
| **Reference** | |
| **Reference**: | Rhoton, S.L., R.A. Perkins, J.F. Braddock, and C. Behr-Andres. 2001. A cold-weather species' response to chemically dispersed fresh and weathered Alaska North Slope crude oil. In: Proceedings, 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC. pp. 1231 – 1236.<br><br>Rhoton, S.L. 1999. Acute toxicity of the oil dispersant Corexit 9500, and fresh and weathered Alaska North Slope crude oil to the Alaskan Tanner crab (C. bairdi), two standard test species, and V. fischeri (Microtox® assay). Masters of Science Thesis, University of Alaska, Fairbanks. |

| | |
|---|---|
| **Test Substance** | |
| **Category Name: CRUDE OIL** | |
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Prudhoe Bay crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The sample of Prudhoe Bay crude oil was described as a "medium light crude" and an EPA standard crude oil. It was reported to contain 23.2% (by weight) of components having a boiling point of 205°C or less. |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |

| | |
|---|---|
| **Year Study Performed** : | 1999 |
| **Method/Guideline Followed**: | Generally followed ASTM E729 and OECD 203 |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Menidia beryllina (inland silversides) |
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static-renewal; daily basis |
| **Test Vessel**: | 400-mL beakers; covered with <20% headspace |
| **Water Media Type**: | Reconstituted saltwater using de-ionized water (≥18 MΩ-cm) and Crystal Sea Marinemix. |
| **Test Concentrations**: | Control and five WAF treatments of increasing loading rate. |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 5.8 – 8.3 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.44 Upper Range : 8.70 |
| **Test Temperature and Units**: | Value:<br><br>Lower Range : 22.0 °C<br>Upper Range : 28.5 °C |

| | |
|---|---|
| **Photo (Light/Dark)**: | 16 h light: 8 h dark<br>Light Intensity = 10 – 20 µE/$m^2$/s |
| **Salinity**: | 19.54 – 21.24 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Water accommodated fractions of Prudhoe Bay crude oil were independently created by adding a measured mass of oil to 3.5 L saltwater in a 4-L aspirator bottle. The WAF was created using a standardized method of low-energy mixing such that no vortex was created (0% water depth). Headspace in the aspirator bottle was approximately 25%, and all bottles were covered with aluminum foil. The temperature at which the WAFs were created was regulated at the testing temperature of 25°C. The WAFs were mixed for 24 hours, allowed to stand for 5 minutes and then collected for chemical analysis and immediate dispensing to the test vessels. Each WAF was taken from the bottle 90% of the water depth through the aspirator bottle's sampling port. Each control and WAF treatment was replicated three times.<br><br>Test vessels were 400-mL polypropylene beakers. The volume held in each beaker was not stated, but it was noted that the headspace was kept to <20%. Beakers were covered with a glass watchglass. Every 24 hours, 90% of the test solutions were decanted and fresh WAF was replaced. A low aeration rate (1.68 – 3.35 $cm^3$/min) was used to maintain dissolved oxygen.<br><br>The test animals used in the study were larval M. beryllina purchased from Aquatic Biosystems, Inc. (Ft. Collins, CO). They were acclimated for two days prior to test initiation. Test animals were 12 days old at test initiation. They were fed 1 mL of satwater-rinsed, concentrated, newly-hatched brine shrimp nauplii (Artemia, approximately 100 Artemia per animal) once or twice daily prior to and during the test.<br><br>WAF solutions were analyzed using gas chromatography/flame ionization detection (GC-FID) by US EPA SW-846 methods 5030, 8000B, and 8021B, and ADEC method AK101 and AK102. Solutions were measured for total volatile organic analytes (VOA; range defined as C6-C9) and total petroleum hydrocarbon content (TPH; range defined as C10-C36). The sum of the two fractions was calculated and termed the total hydrocarbon content (THC). Measured values of VOA were determined on a composite sample of WAFs collected from days 1 – 4. TPH was measured only from the initial WAF. This was adopted after verifying that the TPH concentration in each WAF was consistently low in relationship to VOA regardless of the loading rate. This was due to the limited solubility of the C10 – C36 hydrocarbons. |
| **Limit Test**: | N/A |

## Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | 14.81 | | mg/L | death | Measured THC |
| 96 | Hours | LL | 50 | = | 4965 | | mg/L | death | WAF loading rate |

| | |
|---|---|
| **Results Remarks**: | The test endpoint was presented based on the measured total hydrocarbon content (sum of VOA and TPH) and the WAF loading rate. Because aeration was applied to each test chamber, there was a potential for loss of the volatile fraction. This would underestimate the degree of toxicity because exposures were not constant over time. To assess the degree of underestimation of the toxicity values, an analysis of the change in VOA concentrations over time was made. A series of samples were collected and analyzed for VOA from the high and low WAF concentrations during the first 24 hours of a simulated exposure test. The VOA would be expected to be the fraction most affected by aeration. The measured concentrations were plotted against time and the area under the curve (AUC) was calculated. The AUC for the measured concentrations was compared against the AUC for a theoretical constant exposure. The VOA concentrations in the WAFs declined to near detection limits in approximately 12 hours, causing the AUC to be 90% less than the theoretical exposure. Although test solutions were renewed at 24-h intervals during the 96-hour test, these data indicate that the measured endpoints are likely to underestimate the toxicity that would be expected had constant exposures been maintained. |
| **Reliability/Data Quality** | |
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. Although solutions were renewed daily, the use of aeration likely caused some loss of volatile components. |
| **Key Study Sponsor Indicator**: | |
| **Reference** | |
| **Reference**: | Rhoton, S.L., R.A. Perkins, J.F. Braddock, and C. Behr-Andres. 2001. A cold-weather species' response to chemically dispersed fresh and weathered Alaska North Slope crude oil. In: Proceedings, 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC. pp. 1231 – 1236.<br><br>Rhoton, S.L. 1999. Acute toxicity of the oil dispersant Corexit 9500, and fresh and weathered Alaska North Slope crude oil to the Alaskan Tanner crab (C. bairdi), two standard test species, and V. fischeri (Microtox® assay). Masters of Science Thesis, University of Alaska, Fairbanks. |

| | |
|---|---|
| **Test Substance** | |
| **Category Name: CRUDE OIL** | |
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |

| | |
|---|---|
| **Test Substance** : | Bass Strait crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |

## Method

| | |
|---|---|
| **Year Study Performed** : | 2002 |
| **Method/Guideline Followed**: | Other |
| | |
| **Deviations from Method/Guideline** : | N/A |
| **Species**: | Melanotaenia fluviatilis (crimson-spotted rainbowfish) |
| | |
| **GLP**: | Not stated |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Renewal (daily renewal of 50% volume of test solution) |
| **Test Vessel**: | Not stated |
| **Water Media Type**: | Freshwater |
| **Test Concentrations**: | 0 (control), 5, 10, 20, 40, and 80% water soluble fraction of crude oil |
| **Nominal and Measured Concentrations**: | 0 (control), 0.2, 0.4, 0.7, 1.4, 2.7 mg/L |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: none | |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |

| | |
|---|---|
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 7.3 mg/L |
| **pH Value**: | Value: 7.2<br>Lower Range : Upper Range : |
| **Test Temperature and Units**: | Value: 24.4<br>Lower Range :<br>Upper Range : |
| **Photo (Light/Dark)**: | Not stated |
| **Salinity**: | N/A |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | A water accommodated fraction (WAF) of Bass Strait crude oil was prepared at a oil to water ratio of 1:9 (v/v). The WAF was made by mixing oil and water for 24 hours. The solution was allowed to settle for 1 hour and the aqueous fraction was collected and diluted to the fractions used in testing. Every 24 hours of the test, a 50% volume of each test solution was removed and replaced.<br><br>At the beginning of the test, 250 mL of each exposure solution was extracted with dichloromethane and the total petroleum hydrocarbons was measured by gas chromatographic analyses. |
| **Limit Test**: | N/A |

## Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 24 | Hours | LC | 50 | = | 4.48 | | mg/L | Death | Measured total petroleum hydrocarbons |
| 48 | Hours | LC | 50 | = | 3.38 | | mg/L | Death | Measured total petroleum hydrocarbons |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 72 | Hours | LC | 50 | = | 2.10 | | mg/L | Death | Measured total petroleum hydrocarbons |
| 96 | Hours | LC | 50 | = | 1.28 | | mg/L | Death | Measured total petroleum hydrocarbons |
| | | | | | | | | | |

| | |
|---|---|
| **Results Remarks**: | Loss of dissolved hydrocarbons was mitigated by renewing 50% volumes of each exposure solution at 24-h cycles. However, the test vessels were not described, and it is unknown whether or not sealed vessels were used. Therefore, it is possible that some hydrocarbon loss from the solutions may have occurred during the intervals between renewals. |
| **Reliability/Data Quality** | |
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. Some lack of detail in the reported methodology prevented a thorough understanding of the exposure conditions. |
| **Key Study Sponsor Indicator**: | |
| **Reference** | |
| **Reference**: | Pollino, C.A., and D.A. Holdway. 2002. Toxicity testing of crude oil and related compounds using early life stages of the crimson-spotted rainbowfish (Melanotaenia fluviatilis). Ecotoxicol. Environ. Safety 52:180-189. |

| | |
|---|---|
| **Test Substance** | |
| **Category Name: CRUDE OIL** | |
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Louisiana Sweet crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | Non-weathered, Louisiana sweet crude oil, lot # WP 681 purchased from RT Corporation, Laramie, WY |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2010 |
| **Method/Guideline Followed**: | US EPA 821-R-02-012, Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. |
| | |
| **Deviations from Method/Guideline** : | The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. |

| | |
|---|---|
| **Species**: | Menidia beryllina (inland silversides) |
| | |
| **GLP**: | Yes |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static |
| **Test Vessel**: | 1-L beakers containing 1 L of test solution |
| **Water Media Type**: | Natural seawater, filtered and adjusted to a salinity of 20 parts per thousand |
| **Test Concentrations**: | 6 concentrations, with the highest being 100% WAF |
| **Nominal and Measured Concentrations**: | The 100% WAF contained 2.9 mg TPH/L |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | |
| **pH Value**: | Value:<br>Lower Range : Upper Range : |
| **Test Temperature and Units**: | Value: 25°C<br>Lower Range :<br>Upper Range : |
| **Photo (Light/Dark)**: | 16 h light: 8 h dark |
| **Salinity**: | 20 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | A water accommodated fraction of Louisiana Sweet crude oil was made by mixing 25 g/L oil:water (=1:40 ratio) in a glass aspirator bottle. The bottle was filled |

| | |
|---|---|
| | with 19 L of seawater, leaving a 20% headspace above the liquid. Oil was added along with a magnetic stir bar. The solutions were stirred by on a magnetic plate. Stirring was adjusted to achieve a vortex of 25% of the total volume. Bottles were securely covered and the solutions were mixed for 18 hours then allowed to settle for 6 hours. The WAF was removed from the bottom of the bottle without disturbing the surface oil and was used for chemical analysis and for toxicity testing.<br><br>Three replicate test vessels were used at each exposure level. Test organisms were randomly assigned with each replicate receiving 10 animals for a total of 30 animals per treatment. One-liter beakers holding 1L of exposure solution was used for each replicate. The test temperature was maintained at 25°C. All vessels were continuously aerated at a rate of 100 bubbles/min.<br><br>The test animals used in the study were larval M. beryllina purchased from Aquatic Biosystems, Inc. (Ft. Collins, CO). The animals were held a minimum of two days prior to testing. Culture, holding, and testing used the same salinity and temperature regimes. Test animals were 11 or 14 days old at test initiation.<br><br>Concentrations of total dissolved petroleum hydrocarbons were made by taking a 1-L sample of the WAF and extracting the total sample with hexane. The hexane fraction was reduced to 1 mL and analyzed by gas chromatography (GC) and flame ionization detection (FID). The method followed EPA SW-846, Method 8015B-DRO. |
| **Limit Test**: | N/A |

## Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | > | 2.9 | | mg/L | death | Measured TPH |
| | | | | | | | | | |

| | |
|---|---|
| **Results Remarks**: | Measured concentrations of TPH were made at the beginning of the test, and reflect conditions at that time. Because solutions were aerated during the test period, and no further analyses were done, it is not known what the exposure conditions were following the initiation of the test.<br><br>The mortality of M. beryllina did not exceed 7% at the highest test level (100% WAF); thus the test endpoint was reported as being >2.9 mg TPH/L, the concentration measured at the beginning of the test.<br><br>The test design was anticipated to reflect exposure conditions during a spill event of crude oil in which a natural weathering process was permitted. Thus, solutions were not renewed and allowed to be exposed to the air in order for the natural physical/chemical characteristics of the oil's hydrocarbon constituents to influence the exposure regime. |

## Reliability/Data Quality

| | |
|---|---|
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization. |
| **Key Study Sponsor Indicator**: | |
| **Reference** | |
| **Reference**: | Hemmer, M.J., M.G. Barron, and R.M. Greene. 2010. Comparative toxicity of Louisiana Sweet crude oil (LSC) and chemically dispersed LSC to two Gulf of Mexico aquatic test species. U.S. EPA, National Health and Environmental Effects Research Laboratory. Report posted to EPA web site, URL: www.epa.gov/bpspill/reports/phase2dispersant-toxtest.pdf |

| | |
|---|---|
| **Test Substance** | |
| **Category Name: CRUDE OIL** | |
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Arabian medium crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The test oil was weathered, having a final volume 30 – 35% less than fresh oil volume. The weathered oil had the following characteristics:<br><br>Specific gravity..... 0.9129<br>API gravity ..... 23.5<br>Reid vapor pressure ..... 2.1 KPa<br>Viscosity @ 15..... 102.4 CST<br>Viscosity @20 ..... 80.7CST<br>Pour point..... -14°C<br>Sulfur content ..... 2.96 weight % |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2001 |
| **Method/Guideline Followed**: | Other; CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) |
| **Deviations from Method/Guideline** : | |
| **Species**: | Menidia beryllina (inland silversides) |
| **GLP**: | |

| | |
|---|---|
| **Analytical Monitoring** : | Yes |
| **Test Type**: | CROSERF flow-through design |
| **Test Vessel**: | 200-mL chambers; exposure system used cited Singer et al. (1993) |
| **Water Media Type**: | seawater, filtered and adjusted to a salinity of 20 parts per thousand |
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | Mean measured (Test 1): 0 (control), 2.5, 5.4, 6.9, 9.0, and 14.5 mg TPH/L<br>Mean measured (Test 2): 0 (control), 11.6, 14.9, 17.2, 18.9, and 32.3 mg TPH/L |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 6.6 – 8.7 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.3 Upper Range : 7.5 |
| **Test Temperature and Units**: | Value:<br>Lower Range : 23°C<br>Upper Range : 26°C |
| **Photo (Light/Dark)**: | 16 h light: 8 h dark |
| **Salinity**: | 20 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Water accommodated fractions of Arabian Medium crude oil were independently created by adding a measured mass of oil via a gas tight syringe to either 2- or 4-L glass aspirator flasks, giving loading in units of mg oil/L. The sidearm of each flask was closed off with a short length of silicone tubing and a clamp. The flasks were sealed with Teflon stoppers, and placed on magnetic stir plates. The speed was adjusted to zero vortex. WAF solutions were mixed for approximately 48 hours at room temperature (25±2°C). WAFs were immediately drawn from the bottom of the mixing flasks and placed in exposure chambers. |

| | |
|---|---|
| | The spiked exposure tests used a closed flow-through system (Singer et al., 1993) that employed 200-mL vessels to hold the test organisms. The spiked exposure simulated the concentrations marine animals would be exposed to beneath a migrating oil slick; a high initial exposure followed by declining concentrations as the slick advects and disperses.<br><br>Menidia were obtained from Charles Rivers, Inc., and were 7 days old. Organisms were acclimated for 3 days in a 40-L glass aquarium with salinity-adjusted seawater to 20 parts per thousand. The organisms were fed *Artemia* sp. nauplii *ad libitum*.<br><br>Definitive mortality observations were made after the 96-hour exposure. Mortality was based on the number of organisms added to the chamber minus the number of live organisms recovered. Dead organisms were those that showed no response to gentle prodding. LC50 values were calculated using ToxCalc 5.0 software package.<br><br>Samples of the fresh WAF solutions were taken at the beginning of each test and analyzed for Total Petroleum Hydrocarbons (TPH). This was defined by CROSERF as the resolved hydrocarbons ranging from C10 – C36 (Coelho and Aurand, 1997). This analysis included liquid-liquid extraction with methylene chloride. The gas chromatography-mass spectrometry (GC-MS) analysis was conducted on a HP5890 II GC coupled to a HP5972A MS. Toxicity results are expressed the initial measured TPH concentrations.<br><br>Two independent tests were run, with the endpoints provided in the following table. |
| **Limit Test**: | N/A |

### Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | > | 14.5 | | mg/L | death | Measured TPH |
| 96 | hours | LC | 50 | > | 32.3 | | mg/L | death | Measured TPH |

**Results Remarks**:

The following dose-response pattern was observed:

| Initial Conc mg/L | Number Surviving | Surviving/ Total | % Survival | |
|---|---|---|---|---|
| **TEST 1** | | | | |
| 0 (control) | 15 | 15/15 | 100 | |
| 2.5 | 15 | 15/15 | 100 | |
| 5.4 | 15 | 15/15 | 100 | LC50 >14.5 mg/L |
| 6.9 | 15 | 15/15 | 100 | |

| | | | | | |
|---|---|---|---|---|---|
| | 9.0 | 15 | 15/15 | 100 | |
| | 14.5 | 15 | 15/15 | 100 | |
| | **TEST 2** | | | | |
| | 0 (control) | 15 | 15/15 | 100 | |
| | 11.6 | 15 | 15/15 | 100 | |
| | 14.9 | 15 | 15/15 | 100 | LC50 > 32.3 mg/L |
| | 17.2 | 15 | 15/15 | 100 | |
| | 18.9 | 15 | 15/15 | 100 | |
| | 32.3 | 15 | 15/15 | 100 | |

| **Reliability/Data Quality** | |
|---|---|
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization. |
| **Key Study Sponsor Indicator**: | |

| **Reference** | |
|---|---|
| **Reference**: | Fuller, C. and J. Bonner. 2005. Results of the Cooperative API/Texas Testing Program. Section 6 in: Aurand, D. and G. Coelho (eds.). Cooperative aquatic toxicity testing of dispersed oil and the "Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF)." Technical Report 07-03. Ecosystem Management & Associates, Inc. Lusby, Maryland. 105 pp. |

| **Test Substance** | |
|---|---|
| **Category Name: CRUDE OIL** | |
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Arabian medium crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The test oil was weathered, having a final volume 30 – 35% less than fresh oil volume. The weathered oil had the following characteristics:<br><br>Specific gravity..... 0.9129<br>API gravity ..... 23.5<br>Reid vapor pressure ..... 2.1 KPa<br>Viscosity @ 15..... 102.4 CST<br>Viscosity @20 ..... 80.7CST<br>Pour point..... -14°C<br>Sulfur content ..... 2.96 weight % |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2001 |

| | |
|---|---|
| **Method/Guideline Followed**: | Other; CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) |
| **Deviations from Method/Guideline** : | |
| **Species**: | Cyprinodon variegatus (sheepshead minnow) |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | CROSERF flow-through design |
| **Test Vessel**: | 200-mL chambers; exposure system used cited Singer et al. (1993) |
| **Water Media Type**: | seawater, filtered and adjusted to a salinity of 20 parts per thousand |
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | Initial measured (Test 1): 0 (control), 2.537, 2.851, 2.972, 3.825, and 6.133 mg TPH/L<br>Initial measured (Test 2): 0 (control), 1.768, 2.615, 4.82, 4.724, and 5.736 mg TPH/L |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 6.6 – 8.7 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.3 Upper Range : 7.5 |
| **Test Temperature and Units**: | Value:<br>Lower Range : 23°C<br>Upper Range : 26°C |
| **Photo (Light/Dark)**: | 16 h light: 8 h dark |
| **Salinity**: | 20 parts per thousand |
| **TOC**: | |

| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: | | |
|---|---|---|---|

| | |
|---|---|
| **Method/Guideline**<br>**Test Conditions Remarks**: | Water accommodated fractions of Arabian Medium crude oil were independently created by adding a measured mass of oil via a gas tight syringe to either 2- or 4-L glass aspirator flasks, giving loading in units of mg oil/L. The sidearm of each flask was closed off with a short length of silicone tubing and a clamp. The flasks were sealed with Teflon stoppers, and placed on magnetic stir plates. The speed was adjusted to zero vortex. WAF solutions were mixed for approximately 48 hours at room temperature (25±2°C). WAFs were immediately drawn from the bottom of the mixing flasks and placed in exposure chambers.<br><br>The spiked exposure tests used a closed flow-through system (Singer et al., 1993) that employed 200-mL vessels to hold the test organisms. The spiked exposure simulated the concentrations marine animals would be exposed to beneath a migrating oil slick; a high initial exposure followed by declining concentrations as the slick advects and disperses.<br><br>Test fisn (C. variegates) were obtained from Aquatic Biosystems and were 3 days old. Organisms were acclimated overnight in 20 parts per thousand salinity seawater. The organisms were fed *Artemia* sp. nauplii *ad libitum*.<br><br>Definitive mortality observations were made after the 96-hour exposure. Mortality was based on the number of organisms added to the chamber minus the number of live organisms recovered. Dead organisms were those that showed no response to gentle prodding. LC50 values were calculated using ToxCalc 5.0 software package.<br><br>Samples of the fresh WAF solutions were taken at the beginning of each test and analyzed for Total Petroleum Hydrocarbons (TPH). This was defined by CROSERF as the resolved hydrocarbons ranging from C10 – C36 (Coelho and Aurand, 1997). This analysis included liquid-liquid extraction with methylene chloride. The gas chromatography-mass spectrometry (GC-MS) analysis was conducted on a HP5890 II GC coupled to a HP5972A MS. Toxicity results are based on the initial measured TPH concentrations.<br><br>Two independent tests were run, with the endpoints provided in the following table. |
| **Limit Test**: | N/A |

## Test Results

**NOEC/LOEC/NOELR/LOELR**

| | **Exposure Duration**: | **Exposure Units**: | **Value Description**: | **Value or Lower Range**: | **Upper Range**: | **Units**: | **Basis for Concentration**: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| **Exposure Duration**: | **Exposure Units**: | Type | **%**: | **Value Description**: | **Mean Value or Lower Mean Value**: | **Upper Mean Value**: | **Units**: | **Basis for Effect**: | **Basis for Concentration**: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | > | 6.1 | | mg/L | death | Measured TPH |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | > | 5.7 | | mg/L | death | Measured TPH |

| | |
|---|---|
| **Results Remarks**: | The following dose-response pattern was observed:<br><br>Initial Conc mg/L / Number Surviving / Surviving/ Total / % Survival<br>**TEST 1**<br>0 (control): 14, 14/14, 100<br>2.537: 15, 15/15, 100<br>2.851: 14, 14/15, 93<br>2.972: 15, 15/15, 100<br>3.825: 14, 14/15, 93<br>6.133: 15, 15/15, 100<br>LC50 >6.1 mg/L<br><br>**TEST 2**<br>0 (control): 15, 15/15, 100<br>1.768: 15, 15/15, 100<br>2.615: 15, 15/15, 100<br>4.82: 15, 15/15, 100<br>4.724: 10, 10/15, 100<br>5.736: 15, 15/15, 100<br>LC50 > 5.7 mg/L |
| **Reliability/Data Quality** | |
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization. |
| **Key Study Sponsor Indicator**: | |
| **Reference** | |
| **Reference**: | Fuller, C. and J. Bonner. 2005. Results of the Cooperative API/Texas Testing Program. Section 6 in: Aurand, D. and G. Coelho (eds.). Cooperative aquatic toxicity testing of dispersed oil and the "Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF)." Technical Report 07-03. Ecosystem Management & Associates, Inc. Lusby, Maryland. 105 pp. |

## Test Substance

### Category Name: CRUDE OIL

| | |
|---|---|
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Alaska North Slope crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The crude oil was reported to contain approximately 1/3 (w/w) volatile components; those hydrocarbons with a boiling point of 204°C to 274°C or less. |
| **Category Chemical Result Type** : | Measured |

| | |
|---|---|
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 1998 |
| **Method/Guideline Followed**: | Other; CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Menidia beryllina (inland silversides) |
| | |
| **GLP**: | Not stated |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | CROSERF flow-through design |
| **Test Vessel**: | 200-mL chambers; exposure system used cited Singer, et al. (1996) |
| **Water Media Type**: | Reconstituted saltwater using de-ionized water (≥18 MΩ-cm) and Crystal Sea Marinemix. |
| **Test Concentrations**: | Control and five WAF treatments of increasing loading rate. |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 5.8 – 8.3 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.44 Upper Range : 8.70 |
| **Test Temperature and Units**: | Value: |

| | |
|---|---|
| | Lower Range : 22.0 °C<br>Upper Range : 28.5 °C |
| **Photo (Light/Dark)**: | 16 h light: 8 h dark<br>Light Intensity = 10 – 20 µE/$m^2$/s |
| **Salinity**: | 19.54 – 21.24 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline**<br>**Test Conditions Remarks**: | Water accommodated fractions of Alaska North Slope crude oil were independently created by adding a measured mass of oil to 3.5 L saltwater in a 4-L aspirator bottle. The WAF was created using a standardized method of low-energy mixing such that no vortex was created (0% water depth). Headspace in the aspirator bottle was approximately 25%, and all bottles were covered with aluminum foil. The temperature at which the WAFs were created was regulated at the testing temperature of 25°C. The WAFs were mixed for 24 hours, allowed to stand for 5 minutes and then collected for chemical analysis and immediate dispensing to the test vessels. Each WAF was taken from the bottle 90% of the water depth through the aspirator bottle's sampling port. Each control and WAF treatment was replicated three times.<br><br>Testing of the WAFs used spiked exposures to the animals that employed a flow-through system designed by Singer et al. (1990) and Pace and Clark (1993). The spiked exposure simulated the concentrations marine animals would be exposed to beneath a migrating oil slick; a high initial exposure followed by declining concentrations as the slick advects and disperses.<br><br>The test animals used in the study were larval M. beryllina purchased from Aquatic Biosystems, Inc. (Ft. Collins, CO). They were acclimated for two days prior to test initiation. Test animals were 12 days old at test initiation. They were fed 1 mL of satwater-rinsed, concentrated, newly-hatched brine shrimp nauplii (Artemia, approximately 100 Artemia per animal) once or twice daily prior to and during the test.<br><br>WAF solutions taken at the beginning of the test were analyzed using gas chromatography/flame ionization detection (GC-FID) by US EPA SW-846 methods 5030, 8000B, and 8021B, and ADEC method AK101 and AK102. Solutions were analyzed for total volatile organic analytes (VOA; range defined as C6–C9) and total petroleum hydrocarbons (TPH; range defined as C10–C36. The summation of these is the total hydrocarbon content (THC; C6–C36). Chromatographic measurements were made using a Hewlett Packard 5890 GC/FID with nitrogen carrier gas. Measurements reflected the spiked concentrations at the beginning of the test. |
| **Limit Test**: | N/A |

## Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | 26.36 | | mg/L | death | Measured THC |
| 96 | Hours | LL | 50 | = | 3520 | | mg/L | death | WAF loading rate |

| | |
|---|---|
| **Results Remarks**: | Measured concentrations of the different fractions of dissolved hydrocarbons were made at the beginning of the test, and reflect conditions at that time. Because no further analyses were done, the exposure concentrations following the initiation of the test were not measured.<br><br>The test design was anticipated to reflect exposure conditions during a spill event of crude oil. This scenario included a process of dilution of the dissolved hydrocarbons with time. Thus, dissolved hydrocarbon concentrations were highest at the beginning and were gradually diluted over the course of the test. |

## Reliability/Data Quality

| | |
|---|---|
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization. |
| **Key Study Sponsor Indicator**: | |

## Reference

| | |
|---|---|
| **Reference**: | Rhoton, S.L., R.A. Perkins, J.F. Braddock, and C. Behr-Andres. 2001. A cold-weather species' response to chemically dispersed fresh and weathered Alaska North Slope crude oil. In: Proceedings, 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC. pp. 1231 – 1236.<br><br>Rhoton, S.L. 1999. Acute toxicity of the oil dispersant Corexit 9500, and fresh and weathered Alaska North Slope crude oil to the Alaskan Tanner crab (C. bairdi), two standard test species, and V. fischeri (Microtox® assay). Masters of Science Thesis, University of Alaska, Fairbanks. |

## Test Substance

## Category Name: CRUDE OIL

| | |
|---|---|
| **Category Chemical** : | Crude Oil, CAS# 8002-05-9 |
| **Test Substance** : | Prudhoe Bay crude oil |

| | |
|---|---|
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The sample of Prudhoe Bay crude oil was described as a "medium light crude" and an EPA standard crude oil. It was reported to contain 23.2% (by weight) of components having a boiling point of 205°C or less. |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |

## Method

| | |
|---|---|
| **Year Study Performed** : | 1999 |
| **Method/Guideline Followed**: | Other; CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Menidia beryllina (inland silversides) |
| | |
| **GLP**: | Not stated |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | CROSERF flow-through design |
| **Test Vessel**: | 200-mL chambers; exposure system used cited Singer, et al. (1996) |
| **Water Media Type**: | Reconstituted saltwater using de-ionized water (≥18 MΩ-cm) and Crystal Sea Marinemix. |
| **Test Concentrations**: | Control and five WAF treatments of increasing loading rate. |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |

| | |
|---|---|
| **Dissolved Oxygen**: | 5.8 – 8.3 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.44 Upper Range : 8.70 |
| **Test Temperature and Units**: | Value:<br><br>Lower Range :<br>22.0 °C<br>Upper Range :<br>28.5 °C |
| **Photo (Light/Dark)**: | 16 h light: 8 h dark<br>Light Intensity = 10 – 20 µE/$m^2$/s |
| **Salinity**: | 19.54 – 21.24 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Water accommodated fractions of Alaska North Slope crude oil were independently created by adding a measured mass of oil to 3.5 L saltwater in a 4-L aspirator bottle. The WAF was created using a standardized method of low-energy mixing such that no vortex was created (0% water depth). Headspace in the aspirator bottle was approximately 25%, and all bottles were covered with aluminum foil. The temperature at which the WAFs were created was regulated at the testing temperature of 25°C. The WAFs were mixed for 24 hours, allowed to stand for 5 minutes and then collected for chemical analysis and immediate dispensing to the test vessels. Each WAF was taken from the bottle 90% of the water depth through the aspirator bottle's sampling port. Each control and WAF treatment was replicated three times.<br><br>Testing of the WAFs used spiked exposures to the animals that employed a flow-through system designed by Singer et al. (1990) and Pace and Clark (1993). The spiked exposure simulated the concentrations marine animals would be exposed to beneath a migrating oil slick; a high initial exposure followed by declining concentrations as the slick advects and disperses.<br><br>The test animals used in the study were larval M. beryllina purchased from Aquatic Biosystems, Inc. (Ft. Collins, CO). They were acclimated for two days prior to test initiation. Test animals were 12 days old at test initiation. They were fed 1 mL of satwater-rinsed, concentrated, newly-hatched brine shrimp nauplii (Artemia, approximately 100 Artemia per animal) once or twice daily prior to and during the test.<br><br>WAF solutions taken at the beginning of the test were analyzed using gas chromatography/flame ionization detection (GC-FID) by US EPA SW-846 methods 5030, 8000B, and 8021B, and ADEC method AK101 and AK102. Solutions were analyzed for total volatile organic analytes (VOA; range defined as C6–C9) and total petroleum hydrocarbons (TPH; range defined as C10–C36. The summation of these is the total hydrocarbon content (THC; C6–C36). Chromatographic measurements were made using a Hewlett Packard 5890 GC/FID with nitrogen carrier gas. Measurements reflected the spiked concentrations at the beginning of the test. |
| **Limit Test**: | N/A |

## Test Results

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | >19.86 | | mg/L | death | Measured THC |
| 96 | Hours | LL | 50 | = | >8152 | | mg/L | death | WAF loading rate |

| | |
|---|---|
| **Results Remarks**: | Measured concentrations of the different fractions of dissolved hydrocarbons were made at the beginning of the test, and reflect conditions at that time. Because no further analyses were done, the exposure concentrations following the initiation of the test were not measured.<br><br>The test design was anticipated to reflect exposure conditions during a spill event of crude oil. This scenario included a process of dilution of the dissolved hydrocarbons with time. Thus, dissolved hydrocarbon concentrations were highest at the beginning and were gradually diluted over the course of the test. |

### Reliability/Data Quality

| | |
|---|---|
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization. |
| **Key Study Sponsor Indicator**: | |

### Reference

| | |
|---|---|
| **Reference**: | Rhoton, S.L., R.A. Perkins, J.F. Braddock, and C. Behr-Andres. 2001. A cold-weather species' response to chemically dispersed fresh and weathered Alaska North Slope crude oil. In: Proceedings, 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC. pp. 1231 – 1236.<br><br>Rhoton, S.L. 1999. Acute toxicity of the oil dispersant Corexit 9500, and fresh and weathered Alaska North Slope crude oil to the Alaskan Tanner crab (C. bairdi), two standard test species, and V. fischeri (Microtox® assay). Masters of Science Thesis, University of Alaska, Fairbanks. |

**Type** : static
**Species** : Cyprinodon variegatus (Fish, estuary, marine)

| | | |
|---|---|---|
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | 2900 - 80000 measured/nominal |
| **Analytical monitoring** | : | yes |
| **Method** | : | Procedure as detailed in paper by Anderson (see Reference) |
| **Year** | : | 1974 |
| **GLP** | : | no data |
| **Test substance** | : | South Louisiana and Kuwait crude oils |
| **Method** | : | The test species was the Sheepshead Minnow. Dispersions of two crude oil samples in water were prepared by shaking the constituents together vigorously for 5 minutes on a shaker platform. Fish tests were run at 5 concentrations. Figures quoted are for the loading rates. |
| **Remark** | : | These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the $LC_{50}$ values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '3', not reliable. |
| **Result** | : | The results were as follows:<br>For Kuwait crude:<br>48-hour $LC_{50}$ = 80 000+ mg/l<br>96-hour $LC_{50}$ = 80 000+ mg/l<br>For South Louisiana crude:<br>48-hour $LC_{50}$ = 33 000 mg/l<br>96-hour $LC_{50}$ = 29 000 mg/l<br><br>Parallel tests run with water-soluble fractions (WSF) failed to produce any meaningful results. |
| **Reliability** | : | (3) not reliable |

(2)

| | | |
|---|---|---|
| **Type** | : | static |
| **Species** | : | Fundulus similis (Fish, estuary, marine) |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | 6000 - 14800 measured/nominal |
| **Method** | : | Procedure as detailed in paper by Anderson (see Reference) |
| **Year** | : | 1974 |
| **GLP** | : | yes |
| **Test substance** | : | South Louisiana and Kuwait crude oils |
| **Method** | : | The test species was the Longnose Killifish. Dispersions of two crude oil samples in water were prepared by shaking the constituents together vigorously for 5 minutes on a shaker platform. Fish tests were run at 5 concentrations. Figures quoted are for the loading rates. |
| **Remark** | : | These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the $LC_{50}$ values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '3', not reliable. |
| **Result** | : | The results were as follows: |

For Kuwait crude:
48-hour $LC_{50}$ = 14 800 mg/l
96-hour $LC_{50}$ = 14 800 mg/l
For South Louisiana crude:
48-hour $LC_{50}$ = 6000 mg/l
96-hour $LC_{50}$ = 6000 mg/l

**Reliability** : (3) not reliable

(2)

**Type** : static
**Species** : Menidia beryllina (Fish, estuary, marine)
**Exposure period** : 96 hour(s)
**Unit** : mg/l
**LC50** : 3700 - 9400 measured/nominal
**Analytical monitoring** : no
**Method** : Procedure as detailed in paper by Anderson (see Reference)
**Year** : 1974
**GLP** : yes
**Test substance** : South Louisiana and Kuwait crude oils

**Method** : The test species was the Tidewater Silverside. Dispersions of two crude oil samples in water were prepared by shaking the constituents together vigorously for 5 minutes on a shaker platform. Fish tests were run at 5 concentrations. Figures quoted are for the loading rates.

**Remark** : These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. Since significant evaporative losses of lower hydrocarbons may have occurred, the $LC_{50}$ values are expected to be lower than those cited. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '3', not reliable.

**Result** : The results were as follows:
For Kuwait crude:
48-hour $LC_{50}$ = 15 000 mg/l,
96-hour $LC_{50}$ = 9400 mg/l
For South Louisiana crude:
48-hour $LC_{50}$ = 5000 mg/l
96-hour $LC_{50}$ = 3700 mg/l

**Source** : CONCAWE Bruxelles
**Reliability** : (3) not reliable

(2)

**Type** : static
**Species** : Salmo gairdneri (Fish, estuary, fresh water)
**Exposure period** : 48 hour(s)
**Unit** : mg/l
**Analytical monitoring** : yes
**Method** : Procedure as detailed in paper by Lockhart, Danell and Murray (see Reference)
**Year** : 1987

| | | |
|---|---|---|
| **GLP** | : | yes |
| **Test substance** | : | Norman Wells crude oil |
| **Method** | : | The test species was the Rainbow Trout. A water-soluble fraction (WSF) of the test substance was prepared by adding crude oil to water at a concentration of 12.5 ml/l and stirring for 2 hours. After settling for 72 hours, groups of 5 fish were exposed to solutions containing 20, 30, 40 and 50% WSF. Three series of tests were run:<br>(a) with closed vessels<br>(b) with open vessels, and<br>(c) with aerated vessels. |
| **Remark** | : | These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '2', reliable with restrictions. |
| **Result** | : | The results were as follows:<br>48-hour $LC_{50}$ (open vessel) = 11.6 mg/l<br>48-hour $LC_{50}$ (closed vessel) = 10.4 mg/l<br>based on measurements of dissolved hydrocarbons made at the beginning and end of the 48-hour period. No fish died in the aerated vessels, and hydrocarbons were undetectable in solution at the 48-hour time point in these studies. |
| **Source** | : | CONCAWE Bruxelles |
| **Reliability** | : | (2) valid with restrictions |

(44)

## 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

| **Category Name:** **CRUDE OIL** | |
|---|---|
| **Category Chemical** : | Crude oil, CAS no. 8002-05-9 |
| **Test Substance** : | Arabian medium crude oil |

| | |
|---|---|
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The test oil was weathered, having a final volume 30 – 35% less than fresh oil volume. The weathered oil had the following characteristics:<br><br>Specific gravity .....0.9129<br>API gravity.....23.5<br>Reid vapor pressure.....2.1 KPa<br>Viscosity @ 15 .....102.4 CST<br>Viscosity @20 .....80.7CST<br>Pour point .....-14°C<br>Sulfur content.....2.96 weight % |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2001 |
| **Method/Guideline Followed**: | EPA (1985), Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, EPA/600/4-85/013. |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Mysidopsis bahia (Americamysis bahia) |
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static-renewal; 24-h intervals |
| **Test Vessel**: | 500-mL amber bottles; sealed, no headspace |
| **Water Media Type**: | seawater, filtered and adjusted to a salinity of 20 parts per thousand |
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | Mean measured (Test 1): 0 (control), 1.05, 1.54, 2.85, 3.62, 5.63 mg TPH/L<br>Mean measured (Test 2): 0 (control), 0.63, 0.65, 0.68, 0.80, 1.24 mg TPH/L |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |

| | |
|---|---|
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 5.0 – 7.4 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.1 Upper Range : 7.9 |
| **Test Temperature and Units**: | Value;<br>Lower Range<br>Upper Range : 25°C |
| **Photo (Light/Dark)**: | 16 h light / 8 h dark |
| **Salinity**: | 20 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value:<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline**<br>**Test Conditions Remarks**: | Exposure solutions consisted of independently-made water accommodated fractions (WAF) of the test oil. WAFs were prepared in either 2- or 4-L glass aspirator flasks. The sidearm of each flask was closed off with a short length of silicone tubing and a clamp. Crude oil was added by weight directly onto the pre-measured dilutions water with a gas tight syringe, giving loading in units of mg oil/L water. The flasks were sealed with Teflon stoppers, and placed on magnetic stir plates. The speeds were adjusted to zero vortex. WAF solutions were mixed for approximately 48 hours at room temperature (25±2°C). WAFs were immediately drawn from the bottom of the mixing flasks and placed in exposure chambers. Exposure chambers were 500-mL amber glass jars with Teflon-lined lids. Each jar was filled with respective WAF to within a few millimeters of the rim before organisms were added. Once organisms were added the chambers were sealed with the Teflon-lined lids to prevent loss of volatile components. Every 24 hours, 75% of the solution in each chamber was removed by straining through Nitex (100 micron) mesh to prevent loss of organisms and replaced with fresh test solutions and resealed.<br><br>Mysids were obtained from Charles Rivers, Inc., and were 7 days old. Organisms were acclimated for 3 days in a 40-L glass aquarium with salinity-adjusted seawater to 20 parts per thousand. The organisms were fed *Artemia* sp. nauplii *ad libitum*.<br><br>Definitive mortality observations were made after the 96-hour exposure. Mortality was based on the number of organisms added to the chamber minus the number of live organisms recovered. Dead organisms were those that showed no response to gentle prodding. LC50 values were calculated using ToxCalc 5.0 software package. |

| | |
|---|---|
| | Samples of the fresh WAF solutions were taken at the beginning of each renewal period and analyzed for Total Petroleum Hydrocarbons (TPH) as defined by CROSERF as the resolved hydrocarbons ranging from C10 – C36 (Coelho and Aurand, 1997). This analysis included liquid-liquid extraction with methylene chloride. The gas chromatography-mass spectrometry (GC-MS) analysis was conducted on a HP5890 II GC coupled to a HP5972A MS. Toxicity results are expressed on daily mean TPH concentrations.<br><br>Two independent tests were run, with the endpoints provided in the following table. |
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC:** | | | | | | | |
| **LOEC:** | | | | | | | |
| **NOELR:** | | | | | | | |
| **LOELR:** | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | 0.56 | | mg/L | Death | Measured TPH |
| 96 | hours | LC | 50 | = | 0.67 | | mg/L | Death | Measured TPH |
| | | | | | | | | | |
| | | | | | | | | | |

**Results Remarks**:

The following dose-response pattern was observed in the two tests:

| Mean Conc mg/L | Number Surviving | Surviving/ Total | % Survival | |
|---|---|---|---|---|
| **TEST 1** | | | | |
| 0 (control) | 16 | 16/16 | 100 | |
| 1.05 | 2 | 2/15 | 13 | |
| 1.53 | 1 | 1/15 | 7 | |
| 2.85 | 0 | 0/16 | 0 | LC50 = 0.56 mg/L |
| 3.62 | 0 | 0/17 | 0 | |
| 5.63 | 0 | 0/16 | 0 | |
| **TEST 2** | | | | |
| 0 (control) | 18 | 18/18 | 100 | |
| 0.63 | 12 | 12/16 | 75 | |

| | | | | | |
|---|---|---|---|---|---|
| | 0.65<br>0.68<br>0.80<br>1.24 | 8<br>4<br>7<br>1 | 8/16<br>4/16<br>7/17<br>1/16 | 50<br>25<br>41<br>6 | LC50 = 0.67 mg/L |

**Reliability/Data Quality**

**Reliability**: 2

**Reliability Remarks**: Reliable with restrictions.

**Key Study Sponsor Indicator**:

**Reference**

**Reference**: Fuller, C. and J. Bonner. 2005. Results of the Cooperative API/Texas Testing Program. Section 6 in: Aurand, D. and G. Coelho (eds.). Cooperative aquatic toxicity testing of dispersed oil and the "Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF)." Technical Report 07-03. Ecosystem Management & Associates, Inc. Lusby, Maryland. 105 pp.

| **Category Name: CRUDE OIL** | |
|---|---|
| **Category Chemical** : | Crude oil, CAS no. 8002-05-9 |
| **Test Substance** : | Alaska North Slope crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The crude oil was reported to contain approximately 1/3 (w/w) volatile components; those hydrocarbons with a boiling point of 204°C to 274°C or less. |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 1998 |
| **Method/Guideline Followed**: | Generally followed ASTM E729 and OECD 203 |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Chionocetes bairdi (Tanner crab) |
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static-renewal; 24-hour cycle |

| | |
|---|---|
| **Test Vessel**: | 400-mL beakers; covered with <20% headspace |
| **Water Media Type**: | Reconstituted saltwater using de-ionized water (≥18 MΩ-cm) and Crystal Sea Marinemix. |
| **Test Concentrations**: | Control and five WAF treatments of increasing loading rate |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**□ | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 8.63 – 10.18 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.65 Upper Range : 8.44 |
| **Test Temperature and Units**: | Value;<br>Lower Range<br>Upper Range :<br>4.60°C<br>8.10°C |
| **Photo (Light/Dark)**: | 16 h light / 8 h dark<br>Light Intensity = 10 – 20 μE/$m^2$/s |
| **Salinity**: | 30.77 – 31.97 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value:<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Water accommodated fractions of Alaska North Slope crude oil were independently created by adding a measured mass of oil to 3.5 L saltwater in a 4-L aspirator bottle. The WAF was created using a standardized method of low-energy mixing such that no vortex was created (0% water depth). Headspace in the aspirator bottle was approximately 25%, and all bottles were covered with aluminum foil. The temperature at which the WAFs were created was regulated at the testing temperature of 25°C. The WAFs were mixed for 24 hours, allowed to stand for 5 minutes and then collected for chemical analysis and immediate dispensing to the test vessels. Each WAF was taken from the bottle 90% of the water depth through the aspirator bottle's sampling port. Each control and WAF treatment was replicated three times.<br><br>Test vessels were 400-mL polypropylene beakers. The volume held in |

| | |
|---|---|
| | each beaker was not stated, but it was noted that the headspace was kept to <20%. Beakers were covered with a glass watchglass. Every 24 hours, 90% of the test solutions were decanted and fresh WAF was replaced. A low aeration rate (1.68 – 3.35 cm3/min) was used to maintain dissolved oxygen.<br><br>The test animals used in the study were larval M. beryllina purchased from Aquatic Biosystems, Inc. (Ft. Collins, CO). They were acclimated for two days prior to test initiation. Test animals were 12 days old at test initiation. They were fed 1 mL of satwater-rinsed, concentrated, newly-hatched brine shrimp nauplii (Artemia, approximately 100 Artemia per animal) once or twice daily prior to and during the test.<br><br>WAF solutions were analyzed using gas chromatography/flame ionization detection (GC-FID) by US EPA SW-846 methods 5030, 8000B, and 8021B, and ADEC method AK101 and AK102. Solutions were measured for total volatile organic analytes (VOA; range defined as C6-C9) and total petroleum hydrocarbon content (TPH; range defined as C10-C36). The sum of the two fractions was calculated and termed the total hydrocarbon content (THC). Measured values of VOA were determined on a composite sample of WAFs collected from days 1 – 4. TPH was measured only from the initial WAF. This was adopted after verifying that the TPH concentration in each WAF was consistently low in relationship to VOA regardless of the loading rate. This was due to the limited solubility of the C10 – C36 hydrocarbons. |
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC:** | | | | | | | |
| **LOEC:** | | | | | | | |
| **NOELR:** | | | | | | | |
| **LOELR:** | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | 2.54 | | mg/L | Death | Measured THC |
| 96 | hours | LL | 50 | = | 12.48 | | mg/L | Death | WAF loading rate |
| | | | | | | | | | |
| | | | | | | | | | |

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|---|---|
| **Results Remarks**: | The test endpoint was presented based on the measured total hydrocarbon content (sum of VOA and TPH) and the WAF loading rate. Because aeration was applied to each test chamber, there was a potential for loss of the volatile fraction. This would |

| | |
|---|---|
| | underestimate the degree of toxicity because exposures were not constant over time. To assess the degree of underestimation of the toxicity values, an analysis of the change in VOA concentrations over time was made. A series of samples were collected and analyzed for VOA from the high and low WAF concentrations during the first 24 hours of a simulated exposure test. The VOA would be expected to be the fraction most affected by aeration. The measured concentrations were plotted against time and the area under the curve (AUC) was calculated. The AUC for the measured concentrations was compared against the AUC for a theoretical constant exposure. The VOA concentrations in the WAFs declined to near detection limits in approximately 12 hours, causing the AUC to be 90% less than the theoretical exposure. Although test solutions were renewed at 24-h intervals during the 96-hour test, these data indicate that the measured endpoints are likely to underestimate the toxicity that would be expected had constant exposures been maintained. |

**Reliability/Data Quality**

**Reliability**: 2

**Reliability Remarks**: Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. Although solutions were renewed daily, the use of aeration likely caused some loss of volatile components.

**Key Study Sponsor Indicator**:

**Reference**

**Reference**:
Rhoton, S.L., R.A. Perkins, J.F. Braddock, and C. Behr-Andres. 2001. A cold-weather species' response to chemically dispersed fresh and weathered Alaska North Slope crude oil. In: Proceedings, 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC. pp. 1231 – 1236.

Rhoton, S.L. 1999. Acute toxicity of the oil dispersant Corexit 9500, and fresh and weathered Alaska North Slope crude oil to the Alaskan Tanner crab (C. bairdi), two standard test species, and V. fischeri (Microtox® assay). Masters of Science Thesis, University of Alaska, Fairbanks.

| **Category Name: CRUDE OIL** | |
|---|---|
| **Category Chemical** : | Crude oil, CAS no. 8002-05-9 |
| **Test Substance** : | Alaska North Slope crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The crude oil was reported to contain approximately 1/3 (w/w) volatile components; those hydrocarbons with a boiling point of 204°C to 274°C or less. |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 1998 |
| **Method/Guideline Followed**: | Generally followed ASTM E729 and OECD 203 |
| | |
| **Deviations from Method/Guideline** : | |

| | |
|---|---|
| **Species**: | Mysidopsis bahia (Americamysis bahia) |
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static-renewal; 24-hour cycle |
| **Test Vessel**: | 400-mL beakers; covered with <20% headspace |
| **Water Media Type**: | Reconstituted saltwater using de-ionized water (≥18 MΩ-cm) and Crystal Sea Marinemix. |
| **Test Concentrations**: | Control and five WAF treatments of increasing loading rate |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 4.80 – 8.60 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.50 Upper Range : 8.65 |
| **Test Temperature and Units**: | Value; 23°C<br>Lower Range 29°C<br>Upper Range : |
| **Photo (Light/Dark)**: | 16 h light / 8 h dark<br>Light Intensity = 10 – 20 µE/$m^2$/s |
| **Salinity**: | 17.67 – 23.51 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value:<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Water accommodated fractions of Alaska North Slope crude oil were independently created by adding a measured mass of oil to 3.5 L |

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| | saltwater in a 4-L aspirator bottle. The WAF was created using a standardized method of low-energy mixing such that no vortex was created (0% water depth). Headspace in the aspirator bottle was approximately 25%, and all bottles were covered with aluminum foil. The temperature at which the WAFs were created was regulated at the testing temperature of 25°C. The WAFs were mixed for 24 hours, allowed to stand for 5 minutes and then collected for chemical analysis and immediate dispensing to the test vessels. Each WAF was taken from the bottle 90% of the water depth through the aspirator bottle's sampling port. Each control and WAF treatment was replicated three times.<br><br>Test vessels were 400-mL polypropylene beakers. The volume held in each beaker was not stated, but it was noted that the headspace was kept to <20%. Beakers were covered with a glass watchglass. Every 24 hours, 90% of the test solutions were decanted and fresh WAF was replaced. A low aeration rate (1.68 – 3.35 cm3/min) was used to maintain dissolved oxygen.<br><br>The test animals used in the study were larval M. beryllina purchased from Aquatic Biosystems, Inc. (Ft. Collins, CO). They were acclimated for two days prior to test initiation. Test animals were 12 days old at test initiation. They were fed 1 mL of satwater-rinsed, concentrated, newly-hatched brine shrimp nauplii (Artemia, approximately 100 Artemia per animal) once or twice daily prior to and during the test.<br><br>WAF solutions were analyzed using gas chromatography/flame ionization detection (GC-FID) by US EPA SW-846 methods 5030, 8000B, and 8021B, and ADEC method AK101 and AK102. Solutions were measured for total volatile organic analytes (VOA; range defined as C6-C9) and total petroleum hydrocarbon content (TPH; range defined as C10-C36). The sum of the two fractions was calculated and termed the total hydrocarbon content (THC). Measured values of VOA were determined on a composite sample of WAFs collected from days 1 – 4. TPH was measured only from the initial WAF. This was adopted after verifying that the TPH concentration in each WAF was consistently low in relationship to VOA regardless of the loading rate. This was due to the limited solubility of the C10 – C36 hydrocarbons. |
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC:** | | | | | | | |
| **LOEC:** | | | | | | | |
| **NOELR:** | | | | | | | |
| **LOELR:** | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | = | 2.61 | | mg/L | Death | Measured THC |
| 96 | hours | LL | 50 | = | 160 | | mg/L | Death | WAF loading rate |
| | | | | | | | | | |
| | | | | | | | | | |

**Results Remarks**:

The test endpoint was presented based on the measured total hydrocarbon content (sum of VOA and TPH) and the WAF loading rate. Because aeration was applied to each test chamber, there was a potential for loss of the volatile fraction. This would underestimate the degree of toxicity because exposures were not constant over time. To assess the degree of underestimation of the toxicity values, an analysis of the change in VOA concentrations over time was made. A series of samples were collected and analyzed for VOA from the high and low WAF concentrations during the first 24 hours of a simulated exposure test. The VOA would be expected to be the fraction most affected by aeration. The measured concentrations were plotted against time and the area under the curve (AUC) was calculated. The AUC for the measured concentrations was compared against the AUC for a theoretical constant exposure. The VOA concentrations in the WAFs declined to near detection limits in approximately 12 hours, causing the AUC to be 90% less than the theoretical exposure. Although test solutions were renewed at 24-h intervals during the 96-hour test, these data indicate that the measured endpoints are likely to underestimate the toxicity that would be expected had constant exposures been maintained.

### Reliability/Data Quality

**Reliability**: 2

**Reliability Remarks**: Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. Although solutions were renewed daily, the use of aeration likely caused some loss of volatile components.

**Key Study Sponsor Indicator**:

### Reference

**Reference**:


Rhoton, S.L., R.A. Perkins, J.F. Braddock, and C. Behr-Andres. 2001. A cold-weather species' response to chemically dispersed fresh and weathered Alaska North Slope crude oil. In: Proceedings, 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC. pp. 1231 – 1236.

Rhoton, S.L. 1999. Acute toxicity of the oil dispersant Corexit 9500, and fresh and weathered Alaska North Slope crude oil to the Alaskan Tanner crab (C. bairdi), two standard test species, and V. fischeri (Microtox® assay). Masters of Science Thesis, University of Alaska, Fairbanks.


| Category Name: CRUDE OIL | |
|---|---|
| **Category Chemical** : | Crude oil, CAS no. 8002-05-9 |
| **Test Substance** : | 10 crude oils |

| | |
|---|---|
| **Test Substance Purity/Composition and Other Test Substance Comments** : | |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | |
| **Method/Guideline Followed**: | |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | |
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | |
| **Test Vessel**: | |
| **Water Media Type**: | |
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | 48 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | |

| | |
|---|---|
| **pH Value**: | Value: 8.2<br>Lower Range : Upper Range : |
| **Test Temperature and Units**: | Value; Lower Range Upper Range : 20°C |
| **Photo (Light/Dark)**: | |
| **Salinity**: | |
| **TOC**: | |
| **Water Hardness**: | Value: Lower Range: Upper Range: 240 mg/L |

**Method/Guideline**
**Test Conditions Remarks**:

Water soluble fractions (WSF) were prepared using a ratio of oil to fresh well water of 1:40 (v/v). The WSF were prepared in 2-L Erlenmeyer flasks fitted with Teflon stopcocks near the bottom for drainage. The caps were lined with Teflon and the flasks were covered with foil to minimize photodegradation. After the water and the Teflon-coated stir bar were added, the oil was gently poured onto the surface of the water. The flask was tightly capped and placed on a magnetic stirrer for three days of stirring at approximately 60 rpm so that no vortex was formed on the underside of the oil and no oil emulsification occurred. The temperature at which the WAFs were created was 20°C. The WSF was used within 3 hours of completion of stirring.

Daphnia magna were maintained in accordance with Environment Canada's protocol. Neonate daphnids less than 24-h old were used in testing. They originated from second or subsequent broods of adults approximately 3 weeks old.

Test vessels were 176-mL glass bottles with Teflon-lined caps. Ten daphnids were placed in each bottle with a volume of dilution water. The bottles were topped up to overflowing with the WSF, which was dispensed directly from the Erlenmeyer flask. Bottles were capped immediately with no air space and gently inverted to mix. Bottles holding the daphnids were held at 20°C in a temperature-controlled room with a photoperiod of 16 h light/8 h dark for 48 hours. Light intensity during the daylight period was 800 lux.

At test termination (48 h), observations for mortalities were made by transferring the daphnids out of the test bottles to a watch glass for observation under a stereo-microscope. Death was defined as lack of movement of antennae and lack of heartbeat when observed for 10 seconds.

Analysis of the 100%, 32%, 10% WAFs and control solutions were made at 0 and 48 hours. Extractions of the WSF were made using cyclohexane. Extracts were placed in amber septum bottles for analysis. Analysis of the total petroleum hydrocarbons in the extracts of the WSFs were made by the purge-and-trap gas chromatographic technique. The following instrument conditions were used:

Purge-and-trap conditions:
Purge time: 15 min
Desorb time: 6 min
Bake time: 25 min

| | |
|---|---|
| | GC Programming:<br>40°C for 10 min<br>8°C/min<br>225°C for 10 min<br><br>The GC response was calibrated with saturated solutions of benzene, toluene, ethyl benzene, xylenes, and MTBE. |
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | **Exposure Duration**: | **Exposure Units**: | **Value Description**: | **Value or Lower Range**: | **Upper Range**: | **Units**: | **Basis for Concentration**: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| **LOELR**: | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| **Exposure Duration**: | **Exposure Units**: | Type | **%**: | **Value Description**: | **Mean Value or Lower Mean Value**: | **Upper Mean Value**: | **Units**: | **Basis for Effect**: | **Basis for Concentration**: |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
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**Results Remarks**:

The 48-hour toxicity tests resulted in the following EC50 values, mg/L dissolved hydrocarbons.

| | 48-h EC50, mg/L (95% CI) |
|---|---|
| Pitas Point Crude Oil | 5.9 (1.3 – 8.6) |
| Oseberg Crude Oil | 13.3 (2.6 – 31) |
| Hondo Crude Oil | 11.8 |
| Dos Cuadras Crude Oil | 4.6 (2.7 – 7.8) |
| Carpinteria Crude Oil | 5.5 (2.3 – 8.2) |
| BCF 24 Crude Oil | 10.6 (8.4 – 15.0) |
| Santa Crude Oil | 7.5 |
| Port Hueneme Crude Oil | >1.07* |
| Beta Crude Oil Clara Crude Oil | >4.4* |
| Sockeye | 12.1 |

| | |
|---|---|
| | * Values greater than indicate toxicity at 100% WSF was insufficient to calculate an EC50.<br>The GC analysis of the WSF samples measured petroleum hydrocarbons based on BTEX, and no indication of the presence of the low molecular weight PAHs or other fractions was available from the analysis. Therefore, the EC values may be slightly lower than if additional hydrocarbons were captured by the measurement method. |

**Reliability/Data Quality**

**Reliability**: 2

**Reliability Remarks**: Reliable with restrictions. The analytical method used to measure the dissolved hydrocarbons was based on BTEX, and may not have captured the total possible dissolved hydrocarbon constituents in the WSF.

**Key Study Sponsor Indicator**:

**Reference**

**Reference**: Environment Canada. 1994. The comparative toxicity of crude and refined oils to Daphnia magna. Report Series EE-152. Environmental Technology Centre, Emergencies Science Division, Environment Canada. 23 pp.

| **Category Name:** | **CRUDE OIL** |
|---|---|
| **Category Chemical** : | Crude oil, CAS no. 8002-05-9 |
| **Test Substance** : | West Texas Sour crude oil<br>West Texan Intermediate crude oil<br>Iranian Light crude oil<br>Waxy Light Heavy Blend crude oil<br>Arabian Light crude oil<br>Arabian Medium crude oil<br>Empire crude oil<br>Maya crude oil<br>Sumatran Heavy crude oil<br>Belridge Heavy crude oil<br>Sumatran Light crude oil<br>Udang crude oil<br>Boscan crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 1994 |

| | |
|---|---|
| **Method/Guideline Followed**: | Other |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Daphnia magna |
| | |
| **GLP**: | Not stated |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static |
| **Test Vessel**: | 176-mL glass bottles with Teflon-lined caps; sealed with no headspace |
| **Water Media Type**: | Well water |
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | 48 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | |
| **pH Value**: | Value: 8.2<br>Lower Range : Upper Range : |
| **Test Temperature and Units**: | Value; 20°C<br>Lower Range<br>Upper Range : |
| **Photo (Light/Dark)**: | |

| | |
|---|---|
| **Salinity**: | |
| **TOC**: | |
| **Water Hardness**: | Value: 240 mg/L<br>Lower Range:<br>Upper Range: |

**Method/Guideline**
**Test Conditions**
**Remarks**:

Water soluble fractions (WSF) were prepared using a ratio of oil to fresh well water of 1:40 (v/v). The WSF were prepared in 2-L Erlenmeyer flasks fitted with Teflon stopcocks near the bottom for drainage. The caps were lined with Teflon and the flasks were covered with foil to minimize photodegradation. After the water and the Teflon-coated stir bar were added, the oil was gently poured onto the surface of the water. The flask was tightly capped and placed on a magnetic stirrer for three days of stirring at approximately 60 rpm so that no vortex was formed on the underside of the oil and no oil emulsification occurred. The temperature at which the WAFs were created was 20°C. The WSF was used within 3 hours of completion of stirring.

Daphnia magna were maintained in accordance with Environment Canada's protocol. Neonate daphnids less than 24-h old were used in testing. They originated from second or subsequent broods of adults approximately 3 weeks old.

Test vessels were 176-mL glass bottles with Teflon-lined caps. Ten daphnids were placed in each bottle with a volume of dilution water. The bottles were topped up to overflowing with the WSF, which was dispensed directly from the Erlenmeyer flask. Bottles were capped immediately with no air space and gently inverted to mix. Bottles holding the daphnids were held at 20°C in a temperature-controlled room with a photoperiod of 16 h light/8 h dark for 48 hours. Light intensity during the daylight period was 800 lux.

At test termination (48 h), observations for mortalities were made by transferring the daphnids out of the test bottles to a watch glass for observation under a stereo-microscope. Death was defined as lack of movement of antennae and lack of heartbeat when observed for 10 seconds.

Analysis of the 100%, 32%, 10% WAFs and control solutions were made at 0 and 48 hours. Analysis of the total petroleum hydrocarbons in the WSFs were made by the gas chromatographic headspace technique. The following instrument conditions were used:

10 mL water equilibrated at 85°C
1 mL headspace injected onto HP-1 capillary column: 25 m, 0.32 mm ID, 1 µm film; Mass Selective Detector.

The GC response was calibrated with saturated solutions of benzene, toluene, ethyl benzene, xylenes, and MTBE.

| | |
|---|---|
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | **Exposure Duration**: | **Exposure Units**: | **Value Description**: | **Value or Lower Range**: | **Upper Range**: | **Units**: | **Basis for Concentration**: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| **LOELR**: | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
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**Results Remarks**:

The 48-hour toxicity tests resulted in the following EC50 values, mg/L dissolved hydrocarbons.

| | 48-h EC50, mg/L (95% CI) |
|---|---|
| West Texas Sour Crude Oil | 28.7 (19.5 – 60.8) |
| West Texas Intermediate Crude Oil | 12.71 (6.45 – 21.1) |
| Iranian Crude Oil | 12.3 |
| Waxy Light Heavy Blend Crude Oil | 4.79 (3.58 – 6.4) |
| Arabian Light Crude Oil | 11.4 (7.92 – 14.15) |
| Arabian Medium Crude Oil | 7.4 (4.98 – 8.9) |
| Empire Crude Oil | 17.3 |
| Maya Crude Oil | >12.9* |
| Sumatran Heavy Crude Oil | N.D.* |
| Belridge Heavy Crude Oil | >0.07* |
| Sumatran Light Crude Oil | >0.95* |
| Udang Crude Oil | N.D.* |
| Boscan Crude Oil | >1.06* |

* Values greater than indicate toxicity at 100% WSF was insufficient to calculate an EC50. Concentrations represent measured hydrocarbons in 100% WSF.
N.D. = No dissolved hydrocarbons were measured in the WSF. Insufficient mortality occurred to calculate and EC50.

The GC analysis of the WSF samples measured petroleum hydrocarbons based on BTEX, and no indication of the presence of the low molecular weight PAHs or other fractions was available from the analysis. Therefore, the EC values may be slightly lower than if additional hydrocarbons were captured by the measurement method.

**Reliability/Data Quality**

| | |
|---|---|
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The analytical method used to measure the dissolved hydrocarbons was based on BTEX, and may not have captured the total possible dissolved hydrocarbon constituents in the WSF. |
| **Key Study Sponsor Indicator**: | |

**Reference**

| | |
|---|---|
| **Reference**: | Environment Canada. 1994. The comparative toxicity of crude and refined oils to Daphnia magna. Report Series EE-152. Environmental Technology Centre, Emergencies Science Division, Environment Canada. 23 pp. |

| **Category Name: CRUDE OIL** | |
|---|---|
| **Category Chemical** : | Crude oil, CAS no. 8002-05-9 |
| **Test Substance** : | Arabian medium crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | The test oil was weathered, having a final volume 30 – 35% less than fresh oil volume. The weathered oil had the following characteristics:<br><br>Specific gravity..... 0.9129<br>API gravity..... 23.5<br>Reid vapor pressure ..... 2.1 KPa<br>Viscosity @ 15..... 102.4 CST<br>Viscosity @20..... 80.7CST<br>Pour point..... -14°C<br>Sulfur content ..... 2.96 weight % |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2001 |
| **Method/Guideline Followed**: | Other; CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Mysidopsis bahia (Americamysis bahia) |
| | |
| **GLP**: | |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | CROSERF flow-through design |
| **Test Vessel**: | 200-mL chambers; exposure system used cited Singer et al. (1993) |
| **Water Media Type**: | Seawater, filtered and adjusted to a salinity of 20 parts per thousand |
| **Test Concentrations**: | |

| | |
|---|---|
| **Nominal and Measured Concentrations**: | Initial measured (Test 1): 0 (control), 6.6, 8.2, 11.1, 13.9, and 14.3 mg TPH/L<br>Initial measured (Test 2): 0 (control), 2.4, 3.1, 4.7, and 11.6 mg TPH/L |
| **Total Exposure Period**: | 96 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 6.6 – 8.7 mg/L |
| **pH Value**: | Value:<br>Lower Range : 7.3 Upper Range : 7.5 |
| **Test Temperature and Units**: | Value;<br>Lower Range 23°C<br>Upper Range : 26°C |
| **Photo (Light/Dark)**: | 16 h light / 8 h dark |
| **Salinity**: | 20 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value:<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Water accommodated fractions of Arabian Medium crude oil were independently created by adding a measured mass of oil via a gas tight syringe to either 2- or 4-L glass aspirator flasks, giving loading in units of mg oil/L. The sidearm of each flask was closed off with a short length of silicone tubing and a clamp. The flasks were sealed with Teflon stoppers, and placed on magnetic stir plates. The speed was adjusted to zero vortex. WAF solutions were mixed for approximately 48 hours at room temperature (25±2°C). WAFs were immediately drawn from the bottom of the mixing flasks and placed in exposure chambers.<br><br>The spiked exposure tests used a closed flow-through system (Singer et al., 1993) that employed 200-mL vessels to hold the test organisms. The spiked exposure simulated the concentrations marine animals would be exposed to beneath a migrating oil slick; a high initial exposure followed by declining concentrations as the slick advects and disperses.<br><br>Mysids were obtained from Charles Rivers, Inc., and were 7 days old. Organisms were acclimated for 3 days in a 40-L glass aquarium with salinity-adjusted seawater to 20 parts per thousand. The organisms were fed *Artemia* sp. nauplii *ad libitum*. |

| | |
|---|---|
| | Definitive mortality observations were made after the 96-hour exposure. Mortality was based on the number of organisms added to the chamber minus the number of live organisms recovered. Dead organisms were those that showed no response to gentle prodding. LC50 values were calculated using ToxCalc 5.0 software package.<br><br>Samples of the fresh WAF solutions were taken at the beginning of each renewal period and analyzed for Total Petroleum Hydrocarbons (TPH) as defined by CROSERF as the resolved hydrocarbons ranging from C10 – C36 (Coelho and Aurand, 1997). This analysis included liquid-liquid extraction with methylene chloride. The gas chromatography-mass spectrometry (GC-MS) analysis was conducted on a HP5890 II GC coupled to a HP5972A MS. Toxicity results are based on the initial measured TPH concentrations.<br><br>Two independent tests were run, with the endpoints provided in the following table. |
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC:** | | | | | | | |
| **LOEC:** | | | | | | | |
| **NOELR:** | | | | | | | |
| **LOELR:** | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | hours | LC | 50 | > | 14.3 | | mg/L | Death | Measured TPH |
| 96 | hours | LC | 50 | > | 11.6 | | mg/L | Death | Measured TPH |
| | | | | | | | | | |
| | | | | | | | | | |

**Results Remarks**:

The assignment of the LC50 values were made by the reviewer of this study based on the following dose-response patterns given in the report.

The following dose-response pattern was observed in the two tests:

| Mean Conc mg/L | Number Surviving | Surviving/ Total | % Survival |
|---|---|---|---|
| **TEST 1** | | | |
| 0 (control) | 15 | 15/15 | 100 |
| 6.6 | 15 | 15/15 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| | 8.2 | 14 | 14/15 | 93 | |
| | 11.1 | 13 | 13/15 | 87 | LC50 > 14.3 mg/L |
| | 13.9 | 13 | 13/15 | 87 | |
| | 14.3 | 12 | 12/15 | 80 | |
| | **TEST 2** | | | | |
| | 0 (control) | 15 | 15/15 | 100 | |
| | 2.4 | 15 | 15/15 | 100 | |
| | 3.1 | 14 | 14/15 | 93 | LC50 > 11.6 mg/L |
| | 4.7 | 14 | 14/15 | 93 | |
| | 11.6 | 13 | 13/15 | 87 | |

**Reliability/Data Quality**

**Reliability**: 2

**Reliability Remarks**: Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization.

**Key Study Sponsor Indicator**:

**Reference**

**Reference**: Fuller, C. and J. Bonner. 2005. Results of the Cooperative API/Texas Testing Program. Section 6 in: Aurand, D. and G. Coelho (eds.). Cooperative aquatic toxicity testing of dispersed oil and the "Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF)." Technical Report 07-03. Ecosystem Management & Associates, Inc. Lusby, Maryland. 105 pp.

| **Category Name:** CRUDE OIL | |
|---|---|
| **Category Chemical** : | Crude Oil, CAS no. 8002-05-9 |
| **Test Substance** : | Louisiana Sweet crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | Non-weathered, Louisiana sweet crude oil, lot # WP 681 purchased from RT Corporation, Laramie, WY |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2010 |
| **Method/Guideline Followed**: | US EPA 821-R-02-012, Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. |
| | |
| **Deviations from Method/Guideline** : | The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. |

| | |
|---|---|
| **Species**: | Americamysis bahia |
| | |
| **GLP**: | Yes |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | Static |
| **Test Vessel**: | 1-L beakers holding 1 L solution |
| **Water Media Type**: | Natural seawater, filtered and adjusted to a salinity of 20 parts per thousand |
| **Test Concentrations**: | 6 concentrations, with the highest being 100% WAF |
| **Nominal and Measured Concentrations**: | The 100% WAF contained 4.4 mg TPH/L |
| **Total Exposure Period**: | 48 hours |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | |
| **pH Value**: | Value or<br>Lower Range : Upper Range : |
| **Test Temperature and Units**: | Value or<br>Lower Range<br>Upper Range : 25°C |
| **Photo (Light/Dark)**: | 16 h light; 8 h dark |
| **Salinity**: | 20 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | A water accommodated fraction of Louisiana Sweet crude oil was made by mixing 25 g/L oil:water (=1:40 ratio) in a glass aspirator bottle. The bottle was filled with 19 L of seawater, leaving a 20% headspace above the liquid. Oil was added along with a magnetic stir bar. The solutions |

| | |
|---|---|
| | were stirred by on a magnetic plate. Stirring was adjusted to achieve a vortex of 25% of the total volume. Bottles were securely covered and the solutions were mixed for 18 hours then allowed to settle for 6 hours. The WAF was removed from the bottom of the bottle without disturbing the surface oil and was used for chemical analysis and for toxicity testing.<br><br>Three replicate test vessels were used at each exposure level. Test organisms were randomly assigned with each replicate receiving 10 animals for a total of 30 animals per treatment. One-liter beakers holding 1L of exposure solution was used for each replicate. The test temperature was maintained at 25°C. All vessels were continuously aerated at a rate of 100 bubbles/min.<br><br>The test animals used in the study were larval M. beryllina purchased from Aquatic Biosystems, Inc. (Ft. Collins, CO). The animals were held a minimum of two days prior to testing. Culture, holding, and testing used the same salinity and temperature regimes. Test animals were 11 or 14 days old at test initiation.<br><br>Concentrations of total dissolved petroleum hydrocarbons were made by taking a 1-L sample of the WAF and extracting the total sample with hexane. The hexane fraction was reduced to 1 mL and analyzed by gas chromatography (GC) and flame ionization detection (FID). The method followed EPA SW-846, Method 8015B-DRO. |
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| **LOELR**: | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 48 | hours | LC | 50 | = | 2.7 | | mg/L | Death | Measured TPH |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

| | |
|---|---|
| **Results Remarks**: | Measured concentrations of TPH were made at the beginning of the test, and reflect conditions at that time. Because solutions were aerated during the test period, and no further analyses were done, it is not known what the exposure conditions were following the initiation of the test. |

| | |
|---|---|
| | The test design was anticipated to reflect exposure conditions during a spill event of crude oil in which a natural weathering process was permitted. Thus, solutions were not renewed and allowed to be exposed to the air in order for the natural physical/chemical characteristics of the oil's hydrocarbon constituents to influence the exposure regime. |
| **Reliability/Data Quality** | |
| **Reliability**: | 2 |
| **Reliability Remarks**: | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization. |
| **Key Study Sponsor Indicator**: | |
| **Reference** | |
| **Reference**: | Hemmer, M.J., M.G. Barron, and R.M. Greene. 2010. Comparative toxicity of Louisiana Sweet crude oil (LSC) and chemically dispersed LSC to two Gulf of Mexico aquatic test species. U.S. EPA, National Health and Environmental Effects Research Laboratory. Report posted to EPA web site, URL: www.epa.gov/bpspill/reports/phase2dispersant-toxtest.pdf |

| **Category Name: CRUDE OIL** | |
|---|---|
| **Category Chemical** : | Crude Oil, CAS no. 8002-05-9 |
| **Test Substance** : | Alaska North Slope crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | Fresh Alaska North Slope (ANS) crude oil contained approximately 1/3 volatiles (boiling point of less than 204°C) |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2003 |
| **Method/Guideline Followed**: | Other; CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) |
| | |
| **Deviations from Method/Guideline** : | |
| **Species**: | Chionocetes bairdi (Tanner crab) |
| | |

| | |
|---|---|
| **GLP**: | Not stated |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | CROSERF flow-through design |
| **Test Vessel**: | 200-mL chambers; exposure system used cited Singer, et al. (1996) |
| **Water Media Type**: | Natural seawater, 0.5-µm filtered |
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | 8.63 – 10.18 mg/L |
| **pH Value**: | Value or<br>Lower Range : 7.65 Upper Range : 8.44 |
| **Test Temperature and Units**: | Value or<br>Lower Range : 4.60°C<br>Upper Range : 8.10°C |
| **Photo (Light/Dark)**: | No regimented photoperiod was used. |
| **Salinity**: | 30.77 – 31.97 parts per thousand |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Independent water accommodated fractions (WAF) representing each exposure level of ANS crude oil were prepared by mixing the oil and water in an aspirator bottle. The slow mixing, with no visible vortex for 24 hours produced a solution that was largely free of micelles or bulk particles of oil.<br><br>Testing of the WAFs used spiked exposures to the animals that employed a flow-through system designed by Singer et al. (1990) |

| | |
|---|---|
| | and Pace and Clark (1993). The spiked exposure simulated the concentrations marine animals would be exposed to beneath a migrating oil slick; a high initial exposure followed by declining concentrations as the slick advects and disperses.<br><br>Larval Tanner crabs less than 24-h old were used to initiate the test. Prior to and during the test, larval crabs were fed once daily with a solution containing a mixture of diatoms (Chaetocerus calcitrans, Chaetocerus gacile, and Thalassiosira pseudonana).<br><br>WAF solutions taken at the beginning of the test were analyzed using gas chromatography/flame ionization detection (GC-FID) by US EPA SW-846 methods 5030, 8000B, and 8021B, and ADEC method AK101 and AK102. Solutions were analyzed for total volatile organic analytes (VOA; range defined as C6–C9) and total petroleum hydrocarbons (TPH; range defined as C10–C36. The summation of these is the total hydrocarbon content (THC; C6–C36). Chromatographic measurements were made using a Hewlett Packard 5890 GC/FID with nitrogen carrier gas. Measurements reflected the spiked concentrations at the beginning of the test. |
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | Exposure Duration: | Exposure Units: | Value Description: | Value or Lower Range: | Upper Range: | Units: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| **LOELR**: | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| Exposure Duration: | Exposure Units: | Type | %: | Value Description: | Mean Value or Lower Mean Value: | Upper Mean Value: | Units: | Basis for Effect: | Basis for Concentration: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | Hours | LL | 50 | = | 285 | | mg/L | death | WAF loading rate |
| 96 | Hours | LC | 50 | = | 13.37 | | mg/L | death | Volatile organic analytes (C6-C9) |
| 96 | Hours | LC | 50 | = | 0.41 | | mg/L | death | Total petroleum hydrocarbons (C10-C36) |
| 96 | Hours | LC | 50 | = | 13.85 | | mg/L | death | Total hydrocarbons (C6-C36) |
| | | | | | | | | | |

| | |
|---|---|
| **Results Remarks**: | Measured concentrations of the different fractions of dissolved hydrocarbons were made at the beginning of the test, and reflect conditions at that time. Because no further analyses were done, the exposure concentrations following the initiation of the test were not measured.<br><br>The test design was anticipated to reflect exposure conditions during a spill event of crude oil. This scenario included a process of dilution of the dissolved hydrocarbons with time. Thus, dissolved hydrocarbon concentrations were highest at the beginning and were gradually diluted over the course of the test. |
| **Reliability/Data Quality** | |
| **Reliability:** | 2 |
| **Reliability Remarks:** | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization. |
| **Key Study Sponsor Indicator:** | |
| **Reference** | |
| **Reference:** | Perkins, R.A., S. Rhoton, and C. Behr-Andres. 2003. Toxicity of dispersed and undispersed, fresh and weathered oil to larvae of a cold-water species, Tanner crab (C. bairdi), and standard warm water test species. Cold Regions Sci. Technol. 36:129-140.<br><br>Rhoton, S.L. 1999. Acute toxicity of the oil dispersant Corexit 9500, and fresh and weathered Alaska North Slope crude oil to the Alaskan Tanner crab (C. bairdi), two standard test species, and V. fischeri (Microtox® assay). Masters of Science Thesis, University of Alaska, Fairbanks. |

| **Category Name: CRUDE OIL** | |
|---|---|
| **Category Chemical** : | Crude Oil, CAS no. 8002-05-9 |
| **Test Substance** : | Alaska North Slope crude oil |
| **Test Substance Purity/Composition and Other Test Substance Comments** : | Fresh Alaska North Slope (ANS) crude oil contained approximately 1/3 volatiles (boiling point of less than 204°C) |
| **Category Chemical Result Type** : | Measured |
| **Test Substance Result Type**: | Measured |
| **Method** | |
| **Year Study Performed** : | 2003 |
| **Method/Guideline Followed**: | Other; CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) |
| | |

| | |
|---|---|
| **Deviations from Method/Guideline** : | |
| **Species**: | Mysidopsis bahia (Americamysis bahia) |
| | |
| **GLP**: | Not stated |
| **Analytical Monitoring** : | Yes |
| **Test Type**: | CROSERF flow-through design |
| **Test Vessel**: | 200-mL chambers; exposure system used cited Singer, et al. (1996) |
| **Water Media Type**: | Natural seawater |
| **Test Concentrations**: | |
| **Nominal and Measured Concentrations**: | |
| **Total Exposure Period**: | |

| | |
|---|---|
| **Vehicle Used**: | None |
| **Vehicle Name**: | |
| | |
| **Vehicle Amount and Units**: | |
| **Alkalinity**: | |
| **Dissolved Oxygen**: | |
| **pH Value**: | Value or<br>Lower Range : Upper Range : |
| **Test Temperature and Units**: | Value or<br>Lower Range<br>Upper Range : 25°C |
| **Photo (Light/Dark)**: | |
| **Salinity**: | |
| **TOC**: | |
| **Water Hardness**: | Value or<br>Lower Range:<br>Upper Range: |

| | |
|---|---|
| **Method/Guideline Test Conditions Remarks**: | Independent water accommodated fractions (WAF) representing each |

| | |
|---|---|
| | exposure level of ANS crude oil were prepared by mixing the oil and water in an aspirator bottle. The slow mixing, with no visible vortex for 24 hours produced a solution that was largely free of micelles or bulk particles of oil.<br><br>Testing of the WAFs used spiked exposures to the animals that employed a flow-through system designed by Singer et al. (1990) and Pace and Clark (1993). The spiked exposure simulated the concentrations marine animals would be exposed to beneath a migrating oil slick; a high initial exposure followed by declining concentrations as the slick advects and disperses.<br><br>Juvenile mysids, at an age of 6 days and approximately 4 mm in length were used. Animals were obtained from Aquatic Bio Systems (Fort Collins, CO).<br><br>WAF solutions were analyzed using gas chromatography/flame ionization detection (GC-FID). Solutions were analyzed for total volatile organic analytes (VOA; range defined as C6–C9) and total petroleum hydrocarbons (TPH; range defined as C10–C36. The summation of these is the total hydrocarbon content (THC; C6–C36). Chromatographic measurements of THC were made using a Hewlett Packard 5890 GC/FID with nitrogen carrier gas. |
| **Limit Test**: | N/A |

**Test Results**

**NOEC/LOEC/NOELR/LOELR**

| | **Exposure Duration**: | **Exposure Units**: | **Value Description**: | **Value or Lower Range**: | **Upper Range**: | **Units**: | **Basis for Concentration**: |
|---|---|---|---|---|---|---|---|
| **NOEC**: | | | | | | | |
| **LOEC**: | | | | | | | |
| **NOELR**: | | | | | | | |
| **LOELR**: | | | | | | | |

**LC/EC/IC/EL/LL Mean Value**

| **Exposure Duration**: | **Exposure Units**: | Type | **%**: | **Value Description**: | **Mean Value or Lower Mean Value**: | **Upper Mean Value**: | **Units**: | **Basis for Effect**: | **Basis for Concentration**: |
|---|---|---|---|---|---|---|---|---|---|
| 96 | Hours | LL | 50 | = | 654 | | mg/L | death | WAF loading rate |
| 96 | Hours | LC | 50 | = | 7.87 | | mg/L | death | Volatile organic analytes (C6-C9) |
| 96 | Hours | LC | 50 | = | 1.78 | | mg/L | death | Total petroleum hydrocarbons (C10-C36) |
| 96 | Hours | LC | 50 | = | 9.625 | | mg/L | death | Total hydrocarbons (C6-C36) |
| | | | | | | | | | |

| | |
|---|---|
| **Results Remarks**: | Measured concentrations of the different fractions of dissolved hydrocarbons were made at the beginning of the test, and reflect conditions at that time. Because no further analyses were done, the exposure concentrations following the initiation of the test were not measured.<br><br>The test design was anticipated to reflect exposure conditions during a spill event of crude oil. This scenario includes a process of dilution of the dissolved hydrocarbons with time. Thus, dissolved hydrocarbon concentrations were highest at the beginning and were gradually diluted over the course of the test. |
| **Reliability/Data Quality** | |
| **Reliability:** | 2 |
| **Reliability Remarks:** | Reliable with restrictions. The test design did not adhere to typical standard testing techniques that require a continuous and consistent exposure of the test substance. However, given the objective to create a more realistic exposure condition, this test provides valuable information that may be used in a hazard characterization. |
| **Key Study Sponsor Indicator:** | |
| **Reference** | |
| **Reference:** | Perkins, R.A., S. Rhoton, and C. Behr-Andres. 2003. Toxicity of dispersed and undispersed, fresh and weathered oil to larvae of a cold-water species, Tanner crab (C. bairdi), and standard warm water test species. Cold Regions Sci. Technol. 36:129-140. |

| | | |
|---|---|---|
| **Species** | : | Crangon crangon (Crustacea) |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | 27 - 110 measured/nominal |
| **Analytical monitoring** | : | No |
| **Method** | : | Procedure as detailed in paper by Franklin and Lloyd (see Reference) |
| **Year** | : | 1982 |
| **GLP** | : | no data |
| **Test substance** | : | Samples of 11 North Sea crude oils |
| **Method** | : | The test species was the Brown Shrimp. Groups of 20 shrimps were exposed to nominal concentrations in the range 17 to 3400 mg/l of each crude oil in sea water. After addition of the crude oil, the solutions were stirred at a constant rate using a shielded stirrer. Crude oil solutions were renewed after 48 hours. Mortalities were recorded daily. |
| **Remark** | : | These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. Since no analytical determinations were reported, these data are ranked by API crude oil task group as '3', not reliable. |
| **Result** | : | The lowest $LC_{50}$ value was obtained for Brent crude and the highest for Thistle crude. Other North Sea crude oils tested were from the Argyll, Auk, Beryl, Claymore, Ekofisk, Forties, Montrose, Murchison and Piper fields. |
| **Source** | : | CONCAWE Bruxelles |
| **Reliability** | : | (3) not reliable |

(32)

| | | |
|---|---|---|
| **Species** | : | Crangon crangon (Crustacea) |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | 41 - 119 measured/nominal |
| **Analytical monitoring** | : | No |
| **Method** | : | Procedure as detailed in paper by Franklin and Lloyd (see Reference) |
| **Year** | : | 1982 |
| **GLP** | : | no data |
| **Test substance** | : | Samples of 8 Middle Eastern crude oils |
| **Method** | : | The test species was the Brown Shrimp. Groups of 20 shrimps were exposed to nominal concentrations in the range 17 to 3400 mg/l of each crude oil in sea water. After addition of the crude oil, the solutions were stirred at a constant rate using a shielded stirrer. Crude oil solutions were renewed after 48 hours. Mortalities were recorded daily. |
| **Remark** | : | These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. Since no analytical determinations were reported, these data are ranked by API crude oil task group as '', not reliable. |
| **Result** | : | The lowest $LC_{50}$ value was obtained for Abu Dhabi crude and the highest for Iranian Light crude. Other crude oils tested were from Libya, Saudi Arabia, Nigeria, Kuwait, Iraq and Iran (heavy crude). |
| **Source** | : | CONCAWE Bruxelles |
| **Reliability** | : | (3) not reliable |

(32)

### 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

| | | |
|---|---|---|
| **Species** | : | Anabaena doloilum (Algae) |
| **Endpoint** | : | growth rate |
| **Exposure period** | : | 15 day(s) |
| **Unit** | : | mg/l |
| **Analytical monitoring** | : | Yes |
| **Method** | : | Procedure as detailed by Gaur and Singh (see Reference) |
| **Year** | : | 1989 |
| **GLP** | : | no data |
| **Test substance** | : | Assam crude oil |
| **Method** | : | Tests were run using:<br>(a) a water-soluble fraction (WSF) of the test substance, and<br>(b) the test substance in equilibrium with the algal suspension medium.<br>To prepare the WSF, crude oil was added to the sterilized medium in the ratio 1:20, and was stirred in closed bottles for 12 hours. After a 4-hour separation period the aqueous phase was separated and diluted for the tests. In the direct loading tests, crude oil was applied to absorbent pads and these were held in the culture suspension. Analysis was done by gas chromatography, following solvent extraction of the aqueous phase using n-pentane. |
| **Remark** | : | These data are included to provide supporting evidence of the expected cumulative toxicity of the soluble components in crude oil. These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. |

These data are ranked by API crude oil task group as '1', reliable without restrictions.

**Result** : The WSF method gave a mean 15-day $EC_{50}$ of 9.06 mg/l, and the direct loading (or whole oil) method gave a 15-day $EC_{50}$ of 5.73 mg/l, both based on measured dissolved hydrocarbon concentrations in the aqueous phase.

**Source** : CONCAWE Bruxelles

**Reliability** : (1) valid without restriction

(34)

## 4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

### 4.5.1 CHRONIC TOXICITY TO FISH

| | | |
|---|---|---|
| **Study Type** | : | chronic toxicity of water soluble fractions of crude oil to fish |
| **Species** | : | rainbow trout (*Oncorhynchus mykiss*) |
| **Exposure Period** | : | 55 days |
| **Unit** | : | mg/l |
| **Analytical Monitoring** | : | Yes |
| **Method** | : | gas chromatography, micro-extraction. Details of the analytical method are described in Murray and Lockhart (1981). |
| **Year** | : | 1996 |
| **GLP** | : | No |
| **Test Substance** | : | Norman Wells crude oil |
| **Statistical Method** | : | levels of significance (i.e., $p<0.05$) for experimental groups were given but methods were not stated |
| **Result** | : | Analytical data showed the following mean measured concentrations of oil corresponding to their nominal concentrations in the exposure solutions. |

| Nominal NWC, ul/l | Nominal NWC, mg/l | Mean Measured Oil Conc, mg/l (±SD) | | Average % of nominal |
|---|---|---|---|---|
| 30 | 24.9 | 0.15 | (0.06) | 0.60 |
| 90 | 74.7 | 0.39 | (0.16) | 0.52 |
| 300 | 249 | 1.51 | (0.45) | 0.61 |

Nominal NWC mg/l values were calculated based on the density of oil of 830.512 g/l at room temperature. The article did not specify the standards by which measured concentrations were based.

Biological Data - Survival

Survival of fish exposed to NWC was decreased from controls and was related to the exposure concentrations. At the lowest concentration of oil (30 ul/l nominal) little mortality occurred until after day 30 of the test, while approximately 50% of the fish exposed to the middle level (90 ul/l) had died by day 30. All fish exposed to 300 ul/l died within that same period. Survival in the control group was 94% or greater. By the end of the test, approximately 50% of the fish exposed to 30 ul/l had died. No end-of-test survival data were given for the 90 ul/l exposure group. Based on these data, the no observed effect concentration for survival was lower than the lowest concentration used in the test (30 ul/l).

Fish Water Content

| | | |
|---|---|---|
| | | Increased water content was not observed after 30 days except in the 90 ul/l treatment. However, by 55 days, fish exposed to NWC had significantly greater ($p$<0.01) water content than fish from the control group. Water content of treated fish tended to increase with duration of exposure.<br><br>mean carcass water content, %<br>@ day of study<br>Group 30 45 55<br>Control 83.6 84.5 83.2<br>30 84.3 86.9 87.0<br>90 85.8 87.9 91.1<br><br>Fish Growth<br>Growth of fish exposed to NWC was reduced ($p$<0.01) from control fish. The differences in growth rate between control and exposed fish were likely due to differences in feeding behavior. Fish exposed to NWC did not swim normally and spent most of the time at the bottom of the test chamber.<br><br>mean fish length, mm<br>@ day of study<br>Group 30 45 55<br>Control 25.1 26.9 27.7<br>30 24.0 23.3 25.0<br>90 22.2 22.9 22.3 |
| **Test Condition** | **:** | A flow-through exposure system provided three concentrations of Norman Wells crude oil (NWC), three corresponding NWC/dispersant mixtures, one concentration of dispersant alone, and a control. The data reported within this summary are for exposures to NWC in the absence of dispersant. Three nominal concentrations of NWC used in the test were 30 ul/l, 90 ul/l, and 300 ul/l. NWC had a density of 830.512 g/l at room temperature, which gave respective weight/volume concentrations of 24.9 mg/l, 74.7 mg/l, and 249 mg/l. Components of the exposure system were constructed of glass and covered to minimize loss of volatile components. Mixing cells in which dilution water and oil mixed were continually stirred by Teflon-coated magnetic bars. Dissolved oxygen in the test chambers remained above 10 mg/l throughout the experiment, and pH ranged from 7.22 to 7.47. Water flow to the 6-L test chambers of 200 ml/min provided a 95% replacement time of 1.5 h. Weekly water samples were taken from all chambers receiving oil and analyzed by gas chromatography.<br><br>Rainbow trout were obtained at the eyed stage of development from spring Valley Trout Farm, Ltd, Petersburg, Ontario and held in the laboratory at 10°C. The test was initiated with the random placement of 25 2-3 day old trout into each test chamber (reviewer assumes the age to indicate 2-3 day old post-hatch stage). The exposure systems operated in a controlled environment room at 10°C with a photoperiod of 12 h light and 12 h dark. Fish were fed Purina Trout Starter at an approximate rate of 5% estimated body weight every second day, beginning on day 10 of exposure.<br><br>Survival was recorded throughout the exposure and fish were sampled after 30 and 45 days when sufficient numbers survived to permit sampling. All survivors were sampled at termination of exposure after 55 days. Length (from tip of snout to end of caudal peduncle), wet weight, dry weight (after 24 hours at 100°C) and water content as the difference between wet and dry weights were determined. |

| | | |
|---|---|---|
| **Reliability** | : | (2) Reliable with restrictions. The published article was based on sound scientific methods, but lacked the details on the statistical analyses and the basis on which measured concentrations were established. |
| **Source** | : | Lockhart, W.L., D.A. Duncan, B.N. Billeck, and R.A. Danell, and M.J. Ryan. 1996. Chronic toxicity of the water soluble fraction of Norman Wells crude oil to juvenile fish. Bull. Spill Sci. Technol. 1(4):259-262.<br><br>Murray, D.A.J. and W.L. Lockhart. 1981. Microextraction and gas chromatographic analysis of selected petroleum hydrocarbons in water and fish tissue. J. chromat. 212:305-311. |
| | | |

## 4.9 ADDITIONAL REMARKS

**Memo** : Effect of crude and refined oil on fresh and sea water organisms

**Remark** : Burks has extensively reviewed the effects of crude and refined oils on organisms found in fresh and sea water. He noted that where spillages occur the non-mobile species suffer the greatest mortality, whereas fish species can often escape from the affected region. The extent of the initial mortality depends on the chemical nature of the oil, the location, and the physical conditions, particularly the temperature and wind velocity. Most affected freshwater and marine communities recover from the effects of an oil spill within a year. The occurrence of biogenic hydrocarbons in the world's oceans is well recorded. They have the characteristic isoprenoid structure, and measurements made in water columns indicate a background concentration of 1.0 to 10 µl/l. The higher molecular weight materials are dispersed as particles, with the highest concentrations of 20 µl/l occurring in the top 3 mm layer of water.
A wide variation in the response of organisms to oil exposures has been noted. The larvae of fish and crustaceans appear to be most susceptible to the water-soluble fraction of crude oil. Exposures of plankton and algae have indicated that certain species of diatoms and green algae are inhibited, whereas microflagellates are not.
For the most part, molluscs and most intertidal worm species appear to be tolerant of oil contamination.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '4', reliability not assigned

**Source** : CONCAWE Bruxelles
**Reliability** : (4) not assignable

(8)

**Memo** : Effect of crude oil on Lobster larvae

**Remark** : The effects of emulsified South Louisiana crude oil on the development of American Lobster, Homarus americanus, was investigated during the development of the first four larval stages over a 15-day period. Hatched larvae were exposed to concentrations of 0, 0.1 and 1.0 ppm crude oil, six

times per day for periods ranging from 0.8 to 5.6 minutes, using a flow-through system. The tests showed that 1.0 ppm crude oil was a sub-lethal concentration for the lobster larvae and that no significant effects were found at 0.1 ppm. At 0.1 ppm crude oil, the survival rate of the larvae was comparable with that of the controls, but at 1.0 ppm the survival value was about 50% that of the control larvae. Other effects observed at 1.0 crude oil included: (a) lethargy, reduced feeding and lack of the characteristic aggression of the control animals, (b) an increase in development time from 12 to 15 days, and (c) a change in the pigmentation of the larvae from the normal pale blue, almost transparent, color to a sharp red appearance.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '4', reliability not assigned

**Source** : CONCAWE Bruxelles
**Reliability** : (4) not assignable

(31)

**Memo** : Effect of crude oil on Sea urchin eggs

**Remark** : Falk-Petersen has studied the effects of the water-soluble fraction (WSF) of Ekofisk crude oil on the development of the eggs of two sea urchin species, Strongylocentrotus pallidus and S. droebachiensis. The WSF was prepared by shaking Ekofisk crude oil and sea water in the ratio 1:9 for 5 minutes, allowing to separate for 20 hours, and using the aqueous phase for the studies.
Fertilized eggs were exposed to dilutions of the WSF for up to 9 days at 3 to 5 °C. Embryos and larvae were examined regularly by scanning and transmission electron microscopy after fixing in 2% $OsO_4$ in sea water. Concentrations of 30% WSF, corresponding to 13 ppm dissolved oil, did not impair either development or the ultrastructure of the stages from egg to pluteus. However, significant effects on development were found at both 40% WSF (17 ppm oil) and 50% WSF (21 ppm oil). At these levels, the larvae filled with degenerating cells, and differentiation of the intestine and skeletal growth were inhibited compared with the control larvae.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '4', reliability not assigned

**Source** : CONCAWE Bruxelles
**Reliability** : (4) not assignable

(28)

**Memo** : Effect of crude oil on eggs

**Remark** : Crude oil from 4 locations was applied externally at different loadings to batches of 30 fertilized eggs of mallard ducks on day 3 of incubation at 37.5 °C. Eggs were candled each day to determine mortality, and dead embryos examined for abnormalities. On day 18 of incubation, surviving embryos were examined for external malformations. The observed LD50 values for crude oils from Kuwait, Prudhoe Bay, South Louisiana and Texas were 2.2, 8.3, 1.3 and 5.5 µl/egg, respectively. The South Louisiana and Texas crudes both produced significantly reduced growth, both above and below the $LD_{50}$ value; preformed embryos were also found with both these crudes.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '4', reliability not assigned

**Reliability** : (4) not assignable

(37)

**Memo** : Poorly water soluble mixtures

**Remark** : For the assessment of the ecotoxicity of poorly water soluble mixtures of hydrocarbons as found in petroleum products, it is now generally accepted that results should be expressed in terms of the "loading rate". The "loading rate" may be defined as the amount of the product which must be equilibrated with the aqueous test medium in order to produce a specified level of effect. Studies in which the results are expressed in terms of the measured concentrations of hydrocarbons in dilutions of "water soluble fractions (WSF)" do not allow the ecotoxicity of a product to be expressed in terms of the amount of that product required to produce a particular effect and, therefore, such results are not comparable to results obtained with other substances.

These data are also cited in the European Chemicals Bureau IUCLID for CAS 8002-05-9. These data are ranked by API crude oil task group as '1', reliable without restrictions.

**Source** : CONCAWE Bruxelles

**Reliability** : (1) valid without restriction

(13) (35) (79)

## 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 5000 mg/kg bw |
| **Species** | : | Rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | male/female |
| **Number of animals** | : | 5 |
| **Vehicle** | : | None, undiluted |
| **Doses** | : | Single dose: 5000 mg/kg |
| **Year** | : | 1984 |
| **GLP** | : | no data |
| **Test substance** | : | Beryl crude (Light crude oil) |
| **Method** | : | Groups of five male and five female Sprague-Dawley rats were dosed once by oral gavage with the test material at a dose level of 5 g/kg. The animals were observed frequently on the day of treatment and daily thereafter for 14 days. The animals were weighed on the day of treatment and again 7 and 14 days later. |
| **Result** | : | There were no deaths following treatment.<br>During the first week post dosing lacrimation and a discharge covering the perineum (genital area) were observed.<br>At termination of the study all animals appeared healthy and had gained weight.<br>The oral $LD_{50}$ was judged to be greater than 5 g/kg. |
| **Reliability** | : | (2) valid with restrictions<br>Not clear whether this study was carried out to GLP and few experimental data given. Nevertheless, the study is sufficient to demonstrate an $LD_{50}$ of greater than 5 g/kg. |

(55)

**Type** : $LD_{50}$

**Result** : The results of other acute toxicity studies which have been reported are as follows:

| **Sample** | **$LD_{50}$** | **Clinical signs** |
|---|---|---|
| Lost Hills Light (Mobil consolidated report) | | |
| | >5 g/kg | Soft stool<br>Urogenital discharge<br>Decreased fecal output<br>Anal discharge |
| MCSL Crude (midcontinent) Mobil study No. 40971 | | |
| | >5 g/kg | Decreased activity<br>Hunching<br>Discharge/perineal staining |
| Arab Light (Mobil study No. 40961) | | |
| | >5 g/kg | Discharge/perineal staining |
| Belridge heavy (Mobil summarized data) | | |
| | >5 g/kg | Mild gastrointestinal effects |

Smith et al (1981) also demonstrated that three different crude oils (Crude

type not specified) were non-toxic in male mice as follows:

| | |
|---|---|
| Wilmington Crude oil | >16 g/kg |
| Recluse crude oil | >16 g/kg |
| Mixed petroleum crudes | >10 g/kg |

(54) (56) (63) (73) (76)

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | $LD_{50}$ |
| **Value** | : | > 2000 mg/kg bw |
| **Species** | : | Rabbit |
| **Strain** | : | New Zealand white |
| **Sex** | : | male/female |
| **Number of animals** | : | 3 |
| **Vehicle** | : | None, undiluted |
| **Doses** | : | Single dose: 2000 g/kg |
| **Year** | : | 1984 |
| **GLP** | : | no data |
| **Test substance** | : | Beryl Crude (Light crude oil) |
| **Method** | : | The skin was clipped from the trunks of three male and three female New Zealand White rabbits prior to treatment with test material. The skin of the backs of three animals (2 male, 1 female) was abraded and the skin of the remaining animals was left intact. The liquid, undiluted test material was then applied as a single dose of 2 g/kg to the shorn back of all six animals. The test site was covered with a gauze and an occlusive wrap. The animals were also fitted with Elizabethan collars to prevent chewing of the occlusive covering and ingestion of test material. 24 hours after application of the test material the occlusive dressing was removed and any surplus test material was removed from the skin by gently wiping with cotton moistened with physiological saline. The animals were observed frequently on the day of treatment and daily thereafter for 14 days.<br>Two hours after wiping the residual material from the skin (26 hours after treatment), the areas of application were assessed for skin irritation. Further evaluations for skin irritation were also made on the 3rd and 7th days following treatment. |
| **Result** | : | There were no deaths in this study.<br>A few animals had soft stool/diarrhea during the observation period, however, all animals were normal at study termination. At the end of the study five of the six animals had gained weight and one had lost a small amount of weight. [No actual body weight data are provided in the report]. There was no evidence of systemic toxicity during the study although there was some skin reaction at the site of application.<br>Following 24 hours of skin contact, varying degrees (slight, moderate) irritation were observed at the 26 and 72 hour readings. At the end of the first week, the skin response was barely perceptible or absent.<br>The dermal $LD_{50}$ was judged to be greater than 2 g/kg |
| **Reliability** | : | (2) valid with restrictions<br>Not clear whether this study was carried out to GLP and few experimental data given. |

(52)

**Type** : $LD_{50}$

**Result** : Additionally, acute dermal $LD_{50}$s have also been reported for three other light crude oils.
The results are as follows:

| Sample | $LD_{50}$ | Clinical signs |
|---|---|---|
| Lost Hills Light (Mobil Study No. 63831) | | |
| | >2 g/kg | Soft stool |
| | | Decreased food consumption |
| | | Decreased fecal output |
| MCSL Crude (midcontinent) Mobil study No. 40972 | | |
| | >2 g/kg | Diarrhea |
| | | Nasal discharge |
| Arab Light (Mobil study No. 40962) | | |
| | >2 g/kg | No signs of toxicity |
| Belridge Heavy (Mobil summarized data) | | |
| | >2 g/kg | Mild gastrointestinal effects |

(51) (53) (63) (73)

### 5.2.1 SKIN IRRITATION

**Species** : Rabbit
**Concentration** : Undiluted
**Exposure** : Occlusive
**Exposure time** : 24 hour(s)
**Number of animals** : 6
**Vehicle** : Undiluted
**Year** : 1985
**GLP** : no data
**Test substance** : Lost Hills Light Crude

**Method** : Prior to test the hair was clipped from the backs of six New Zealand White rabbits (sex not specified). Three one inch square sites (anterior, mid-dorsal and posterior) on the right side of each animal were lightly abraded. Three similarly orientated sites were left intact on the left side of the animal, thus providing six test sites on each rabbit.
0.5 ml of test material was applied to each test site. The material applied to the anterior and mid-dorsal sites was covered with a Webril patch and these were occluded. The dorsal sites remained open. The rabbits were fitted with collars to prevent ingestion of the test material and chewing of the occluded patches.
Four hours after treatment, the anterior sites were unwrapped. The intact site was immediately examined for evidence of corrosive effects and both sites were then gently wiped with a gauze. Thirty minutes later, the skin was evaluated for irritation. The sites were evaluated again 24 hours after patch removal. 48 hours after application, the 4 hour occluded intact site was re assessed for corrosion. Both sites were scored for irritation 4 hours later. The 4 hour occluded sites were again re-evaluated for irritation on day 3.
24 hours after treatment, the two mid-dorsal sites were unwrapped and

along with the posterior uncovered sites were wiped with cotton. The four sites were evaluated for irritation 2 hours later, again approximately 72 hours later and finally on the 7th day after treatment.

**Result** : 4 Hour occluded sites (DOT, OECD methods)

Mean values (24, 48 & 72 hours) for erythema and edema at the intact sites were 1.69 and 1.3 respectively.
The initial response of the skin to the test material was slight, with little difference in response between intact or abraded sites.

Actual scores were:

| | Intact | | Abraded | |
|---|---|---|---|---|
| | Erythema | Edema | Erythema | Edema |
| 4.5 hrs | 1.0 | 1.2 | 1.0 | 1.3 |
| 28 hrs | 1.2 | 1.0 | 1.0 | 1.0 |
| 52 hrs | 1.7 | 1.3 | 1.7 | 1.3 |
| 76 hrs | 1.8 | 1.5 | 1.8 | 1.7 |
| 7 days | 0.8 | 0.7 | 0.5 | 0.7 |
| 10 days | 0 | 0.3 | 0 | 0 |

24 Hour occluded sites (FHSA method)

Actual scores were:

| | Intact | | Abraded | |
|---|---|---|---|---|
| | Erythema | Edema | Erythema | Edema |
| 26 hrs | 1.7 | 1.7 | 1.7 | 1.7 |
| 72 hrs | 2.3 | 1.5 | 2.2 | 1.7 |
| 7 days | 1.2 | 0.7 | 0.8 | 0.8 |
| 10 days | 0.2 | 0 | 0.2 | 0.2 |

Non-occluded sites

Actual scores were:

| | Intact | | Abraded | |
|---|---|---|---|---|
| | Erythema | Edema | Erythema | Edema |
| 26 hrs | 1.7 | 1.2 | 1.8 | 1.3 |
| 72 hrs | 2.8 | 1.5 | 2.7 | 1.3 |
| 7 days | 1.3 | 0.8 | 1.3 | 0.8 |
| 10 days | 0.3 | 0 | 0.2 | 0 |

**Conclusion** : The classification according to various methods/criteria are summarized as follows:

| Guideline | Score | Rating |
|---|---|---|
| DOT Corrosion | Negative | Non-corrosive |
| EEC (4hr occluded) | Erythema 1.6<br>Edema 1.3 | Non-irritant |
| OSHA PII (4 h occl.) | 2.8 | Non-irritant |
| FHSA PII (24 h occl.) | 3.6 | Non-irritant |

**Reliability** : (1) valid without restriction

(63)

| | | |
|---|---|---|
| **Species** | : | Rabbit |
| **Concentration** | : | Undiluted |
| **Exposure** | : | Occlusive |
| **Exposure time** | : | 24 hour(s) |
| **Number of animals** | : | 6 |
| **Vehicle** | : | Non, undiluted |
| **Year** | : | 1984 |
| **GLP** | : | no data |
| **Test substance** | : | Arab Light crude |

**Method** :

Prior to test the hair was clipped from the backs of six New Zealand White rabbits (sex not specified). Three one inch square sites (anterior, mid-dorsal and posterior) on the right side of each animal were lightly abraded. Three similarly orientated sites were left intact on the left side of the animal, thus providing six test sites on each rabbit.
0.5 ml of test material was applied to each test site. The material applied to the anterior and mid-dorsal sites was covered with a Webril patch and these were occluded. The dorsal sites remained open. The rabbits were fitted with collars to prevent ingestion of the test material and chewing of the occluded patches.
Four hours after treatment, the anterior sites were unwrapped. The intact site was immediately examined for evidence of corrosive effects and both sites were then gently wiped with a gauze. Thirty minutes later, the skin was evaluated for irritation. The sites were evaluated again 24 hours after patch removal. 48 hours after application, the 4 hour occluded intact site was re assessed for corrosion. Both sites were scored for irritation 4 hours later. The 4 hour occluded sites were again re-evaluated for irritation on day 3.
24 hours after treatment, the two mid-dorsal sites were unwrapped and along with the posterior uncovered sites were wiped with cotton. The four sites were evaluated for irritation 2 hours later, again approximately 72 hours later and finally on the 7th day after treatment.

**Result** :

4 Hour Occluded (OECD Method, DOT)

Mean values (24, 48 & 72 hours) for erythema and edema at the intact sites were 0.9 and 0.1 respectively.
The initial response of the skin to the test material was slight. Within 72 hours after treatment, most sites had recovered, with little difference in response between intact or abraded sites.

<u>24 Hour Occluded</u> (FHSA method)
The day following application of the test material a moderate erythema was observed. This subsided during the subsequent days such that there was only barely perceptible irritation at the end of the first week. Abrasion had no effect on either the initial response or the recovery.

<u>Non Occluded</u>
Uncovered treated sites had moderate erythema 24 hours after application of the test material. This response diminished and was virtually absent after one week.
Abrasion had no effect on either the initial response or the recovery.

**Reliability** :

(2) valid with restrictions
A textual description of the results only was available. No actual data were provided in the report.

(60)

| | | |
|---|---|---|
| **Species** | : | Rabbit |
| **Concentration** | : | Undiluted |
| **Exposure** | : | Occlusive |
| **Exposure time** | : | 4 hour(s) |
| **PDII** | : | 1.9 |
| **Result** | : | not irritating |
| **Classification** | : | not irritating |
| **Year** | : | 1997 |
| **Test substance** | : | Belridge heavy crude |

**Result** : Summarized data for Belridge Heavy crude oil are:

| Guideline | Score | Rating |
|---|---|---|
| DOT Corrosion | Negative | Non-corrosive |
| EEC (4hr occluded) | Erythema 0.6 | Non-irritant |
| | Edema 0.8 | |
| OSHA PII (4 h occl.) | 1.4 | Non-irritant |
| FHSA PII (24 h occl.) | 2.1 | Non-irritant |

**Reliability** : (2) valid with restrictions
Data only available in tabular form, but studies were part of a series of studies using same methods, all of which were reliability 1.

(73)

### 5.2.2 EYE IRRITATION

| | | |
|---|---|---|
| **Species** | : | Rabbit |
| **Concentration** | : | Undiluted |
| **Dose** | : | .1 ml |
| **Comment** | : | not rinsed |
| **Number of animals** | : | 6 |
| **Year** | : | 1985 |
| **Test substance** | : | Beryl Crude (Light crude oil) |

**Method** : 0.1 ml of undiluted test material was instilled into one eye of each of six New Zealand White rabbits. The eyes were not washed and were evaluated for irritation at 1, 24, 48 and 72 hours after treatment. Fluorescein was used to aid the evaluation of the 72 hour reading. Treated eyes that stained positively with fluorescein were examined again 7 and 14 days after treatment.

**Result** : The mean irritation scores at each of the observation times were:

| | 1 hr | 24 hr | 48 hr | 72 hr |
|---|---|---|---|---|
| Cornea | 0 | 0 | 0 | 0 |
| Iris | 0 | 0 | 0 | 0 |
| Conjunctivae | 4.0 | 1.7 | 1.3 | 1.0 |

The test material was judged to be non-irritating to the rabbit eye.

**Reliability** : (2) valid with restrictions
The only reservation is that it is not clear whether this study was carried out according to GLP. Otherwise the study and reporting are sound.

(58)

**Species** : Rabbit

**Result** : Eye irritation studies have been reported for three other samples of light crude oil.
There were no effects on either the iris or cornea. The only effects recorded were on the conjunctivae.
The mean irritation scores (conjunctivae only) are as follows:

| Crude type | Result 1 hr | 24 hr | 48 hr | 72 hr | Ref |
|---|---|---|---|---|---|
| MCSL (Midcontinent) | 2.3 | 0.3 | 0 | 0.7 | Mobil 40973 |
| Lost Hills Light | 8.0 | 3.7 | 2.7 | 1.7 | Mobil 63832 |
| Arab Light | 5.3 | 1.3 | 0.7 | 0.3 | Mobil 40963 |
| Belridge Heavy | 0.9 | 0.8 | 0.9 | Mobil 1997 (57) (59) (63) (73) | |

## 5.3 SENSITIZATION

**Type** : Buehler Test
**Species** : guinea pig
**Concentration** : 1st: Induction 15 % occlusive epicutaneous
2nd: Challenge 15 % occlusive epicutaneous
3rd: Challenge 10 % occlusive epicutaneous
**Number of animals** : 20
**Vehicle** : Mineral oil
**Result** : not sensitizing
**Year** : 1991
**GLP** : Yes
**Test substance** : Lost Hills Light Crude

**Method** : Concentrations of test material used in this sensitization study were determined in a pre screening study.

Induction phase
0.4 ml of a 15% w/w concentration of test material in mineral oil was applied using a Hill Top Chamber with a 25 mm Webril swatch to the shorn backs of 10 male and 10 female Guinea pigs. The patch was occluded for six hours. The patches were applied to the same site once per week for 3 weeks. After each 6-hour exposure period, the patches were removed and the skin wiped with gauze moistened with mineral oil. The positive control material (DNCB) was applied at a concentration of 0.05% w/w in 70% ethanol to 5 male and 5 female Guinea pigs. Treatment of the positive control animals was the same as for the test animals, except that the skin was wiped with saline after patch removal.

Challenge
Challenge patch application was performed 14 days after the last induction dose had been applied. Dual challenge patches were applied to fresh application sites of previously shorn skin of the animals. The test material

was applied at concentrations of 10 and 15% w/w in mineral oil. The patches were then occluded for 6 hours.
On the day following challenge patch application, the skin was depilated and 2 hours later and scored for signs of sensitization. The sites were examined after a further 48 hours but this time without depilation.
Naive and positive control animals were challenged with DNCB at a concentration of 0.05% in acetone.

**Result** : The results were as follows:

| | **No. responding** | **Mean erythema score** |
|---|---|---|
| Test material (10% concentration) | | |
| 24 hrs induced | 3/19 | 0.2 |
| 24 hrs control | 0/10 | 0 |
| 48 hrs induced | 3/19 | 0.3 |
| 48 hrs control | 2/10 | 0.2 |
| Test material (15% concentration) | | |
| 24 hrs induced | 2/19 | 0.2 |
| 24 hrs control | 2/10 | 0.2 |
| 48 hrs induced | 1/19 | 0.1 |
| 48 hrs control | 1/10 | 0.1 |
| DNCB positive control | | |
| 24 hrs induced | 5/5 | 3.4 |
| 24 hrs control | 0/5 | 0 |
| 48 hrs induced | 5/5 | 2.6 |
| 48 hrs control | 0/5 | 0 |

Although two test animals responded, the test material was judged to be non-sensitizing, since the test criterion required an mean erythema score of +2 for a positive response.

**Reliability** : (1) valid without restriction

(66)

**Type** : Buehler Test
**Concentration** : 1st: Induction 15 %
2nd: Challenge 10 %
3rd: Challenge 15 %
**Result** : not sensitizing
**Year** : 1991
**Test substance** : Belridge heavy crude

**Result** : A similar Buehler test of Belridge heavy crude oil was also negative.
**Reliability** : (2) valid with restrictions

(65)

## 5.4 REPEATED DOSE TOXICITY

| | | |
|---|---|---|
| **Type** | : | Sub-chronic |
| **Species** | : | Rat |
| **Sex** | : | male/female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Dermal |
| **Frequency of treatm.** | : | 5 days/week for 13 weeks |
| **Doses** | : | 30, 125 and 500 mg/kg/day |
| **Control group** | : | yes, concurrent no treatment |
| **Year** | : | 1992 |
| **GLP** | : | no data |
| **Test substance** | : | Two crude oils |

**Method** :

Two separate but identical studies are reported, one for each of two crude oils (Crude I and Crude II). The methods used were identical for both studies.
Undiluted test material was applied to the shorn unoccluded skin of groups of ten male and ten female Sprague-Dawley rats at doses of 30, 125 and 500 mg/kg/day. Application was once daily, five times each week for 13 weeks. Groups of ten rats of each sex served as untreated controls. Each animal was fitted with an Elizabethan collar to prevent ingestion of the applied test material. At the end of each week residual test material was wiped from the backs of the animals. Animals were observed regularly for clinical signs of toxicity and body weights were recorded weekly. The animals were sacrificed during week 14 of the study after fasting overnight. All animals were necropsied and blood samples were taken for a range of hematological and serum chemistry determinations. A range of organs were weighed and tissues examined histologically.
An additional two groups of ten males were treated at a dose level of 0 and 500 mg/kg/day for an evaluation of male reproductive health.
For these animals testes weight and cauda epididymis weights were recorded. Additionally, the number of sperm and % normal sperm in the cauda were recorded as well as the number of spermatids in the testis.

**Result** :

No animals died in either of the studies and there were no clinical signs of systemic toxicity attributable to either crude oil. Both crude oils caused the same minimal skin irritation (flaking) at the exposure site.

<u>Body weights</u>
Apart from the 500 mg/kg/day group for Crude II that gained less weight than the controls (217g compared to 247g over the course of the study). All other body weight measurements were similar to the respective controls.

Hematology
Significant changes relative to their respective controls only occurred in the highest dose group animals (500 mg/kg/day). The % increases (+) or decreases (-) that occurred in these dose groups are shown in the following table. [Changes are shown to the nearest whole number]

| Parameter | Crude I | | Crude II | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| RBC | -6% | NSD | -8% | -7% |
| Hemoglobin | -6% | NSD | -11% | -8% |
| Hematocrit | -5% | NSD | -9% | -8% |
| Platelets | NSD | NSD | -19% | NSD |

NSD No significant difference

Serum chemistry
Although more parameters were affected in the high dose Crude II females, there was no consistent pattern of change that could be attributed to the two crude oils. The differences %+ or %- are shown in the table below.

| | Dose group (mg/kg/day) | | | | | |
|---|---|---|---|---|---|---|
| | Crude I | | | Crude II | | |
| | 30 | 125 | 500 | 30 | 125 | 500 |
| MALES | | | | | | |
| Calcium | | | -7% | | | |
| Glucose | | +13% | +13% | | | |
| Urea nitrogen | | | +53% | | | |
| Uric acid | | | | | | -27% |
| FEMALES | | | | | | |
| ALT | | | | | | -18% |
| Cholesterol | | | | | +41% | +60% |
| Glucose | +16% | | +20% | | | |
| Potassium | | | -11% | | | -13% |
| Urea nitrogen | | | | | | +31% |
| Uric acid | | | | | | -27% |

Necropsy findings
There were no treatment-related observations at necropsy. With the exception of the liver and thymus, there were no absolute or relative organ weight changes when compared to controls.
The organ weight differences that were observed were confined to the 125 and 500 mg/kg/day groups and are summarized below.

| Organ wt | | Crude I | | Crude II | |
|---|---|---|---|---|---|
| | | 125 | 500 | 125 | 500 |
| MALES | | | | | |
| Liver | (absolute) | | +20% | | +22% |
| | (relative) | | +22% | +18% | +33% |
| Thymus | (absolute) | | | | -41% |
| FEMALES | | | | | |
| Liver | (absolute) | | +13% | | +24% |
| | (relative) | | +12% | | +31% |
| Thymus | (absolute) | | | | -35% |
| | (relative) | | | | -27% |

Histopathology
Changes in the skin included hyperplasia and an associated dermal inflammatory cell infiltration. In general the effects were slightly more severe in the animals treated with Crude I. All male and female animals exposed to Crude I were affected whereas at the lowest dose level 8/10 male and 6/10 females exposed to Crude II were affected.
Other histopathological findings were associated with the bone marrow, Liver, thymus and thyroid although effects generally were greater with crude II than with crude I. The findings are tabulated below

Crude I

| | |
|---|---|
| Bone marrow | No effects in either sex at any dose level |
| Liver | Multifocal, mononuclear cell infiltration in 3 males and 2 females at 500 mg/kg/day.<br>Multifocal hepatocellular vacuolation in 3 females at 500 mg/kg/day only |
| Thymus | Atrophy in one male and two females at 500mg/kg/day only |
| Thyroid | Hypertrophy and hyperplasia of follicular epithelium in some males and females at all dose levels |

Crude II

| | |
|---|---|
| | Bone marrow Increased cellularity in 2 males at each dose level (6 males in highest dose group) and focal necrosis in 2 males in the 50 mg/kg/day group.<br>In the females increased cellularity was observed in 9/10 animals |
| Liver | Hepatocellular vacuolation in one male and one female in the 500 mg/kg/day group.<br>Mononuclear cell infiltration was also observed but in only one male in the highest dose group |
| Thymus | Atrophy was observed in six males and 7 females in the 500 mg/g/day group. |
| Thyroid | Hypertrophy and hyperplasia was seen in a few animals at all dose levels. |

The spermatozoa/spermatid evaluations that were conducted on the separate 500 mg/kg/day group of animals exposed to crude I did not reveal any effects when compared to a control.

**Test substance** **:** Two crude oils were examined in the paper by Feuston et al
The characteristics of the crude oils was reported as:

| **Chemical class** | **Crude I** | **Crude II** |
|---|---|---|
| Nonaromatics | 50.0 | 37.3 |
| <3-Ring PAH | 35.3 | 41.7 |
| 3- to 5- Ring PAH | 10.2 | 15.7 |
| 3- Ring PAH | 5.9 | 8.1 |
| 4- Ring PAH | 2.2 | 4.0 |
| 5- Ring PAH | 2.1 | 3.6 |
| Sulfur-PAC | 2.4 | 2.9 |

| | | |
|---|---|---|
| Nitrogen (N)-PAC | 5.4 | 8.4 |
| Nonbasic N-PAC | 3.8 | 5.9 |
| Total | 103.3 | 106.0 |

From information available in the Mobil report of study No 63834 and other laboratory data, it is clear that Crude I is Lost Hills Light Crude and Crude II is Belridge heavy crude oil.

(30) (72)

**Type** : Sub-acute
**Species** : Mouse
**Sex** : male/female
**Strain** : CD-1
**Route of admin.** : Gavage
**Exposure period** : 5 days
**Frequency of treatm.** : Daily for five days
**Doses** : 2, 4, 8, 12 & 16 ml/kg/day
**Control group** : yes, concurrent no treatment
**Year** : 1990
**GLP** : no data
**Test substance** : Prudhoe Bay Crude oil (Heavy crude oil)

**Method** : Two pilot studies were conducted with groups of 5 male and 5 female mice. In these studies Prudhoe Bay crude oil was administered by gavage at doses of 0, 5 and 10 ml/kg daily for five days. No other experimental details are given. However, the results led the author to conduct four further follow-up studies and these are described below.

Experiment 1 (Three crudes, each at a single dose level)
One of three crude oils or Bunker C oil was administered by gavage, daily at a single dose level of 10 ml/kg for 5 days to groups of 10 male mice. A further group of 10 male mice were intubated each day but no material was administered; these animals served as controls.

Experiment 2 (One crude, single dose level)
Groups of 10 male mice were given either Prudhoe Bay crude oil or mineral oil USP or corn oil at a dose of 10 ml/kg/day for five days. A separate group of 10 controls were intubated but no material was administered. This study was to compare the effect of one crude oil with the effects of two oils expected to be non-toxic.

Experiment 3 (One crude, five dose levels)
Prudhoe bay crude oil was administered to 10 or 11 male mice at dose levels of 2, 4, 8, 12 or 16 ml/kg/day. A further group of 10 male mice were sham treated and served as controls.

A fourth experiment was also carried out which studied the effect of crude oil exposure on vitamin E and selenium-deficient animals. This study is not summarized here.

In all experiments 1-3 the animals were weighed on the day they were first dosed and again prior to necropsy. The differences in weights were recorded as change in body weight during the studies. Necropsy was 24 hours after the 5th dose had been administered.
At necropsy, blood samples were taken for determination of blood cell counts, packed cell volume (PCV), hemoglobin and red cell indices

(MCHC). Blood smears were stained with methylene blue to detect the presence of Heinz bodies in red cells. Liver was removed and weighed whereas the spleen and thymus were weighed after fixation. Portions of all major organs were fixed for subsequent histological examination.

Data were analyzed by analysis of variance and regression procedures of the SAS programs (SAS Institute Inc., Cary, NC)

**Result** : Although several studies were reported, the only studies summarized here are study A in which several crude oils are tested at a single dose level and study C in which PBCO is examined at five different dose levels. Neither information on oils other than crude oil or on the effect of Vitamin E and selenium-depletion are included in this robust summary.

In the pilot studies it was reported that the oils were distasteful to the mice and that after the first dose, administration of test material was difficult. Several mice from each group died from inhalation of oil that occurred during the oral dosing. The author stated that the pilot studies demonstrated a dose related decrease (8-11%) in PCV, a reduction in body weight gain, a 74% increase in liver weight per unit body weight and a 66% reduction in thymus weight. No data are given but this was the reason given for the further studies that were carried out.

Although the studies carried out were separate, the author reported the results together and these are summarized below.

Hematology

It was reported (but no data presented) that in experiment A none of the oils tested resulted in significant changes in PCV, number of red blood cells or whole blood hemoglobin. The following table summarizes the hematological results obtained with PBCO only.

| Study | No of mice | Daily dose PBCO (ml/kg/day) | PCV | MCHC (g/l) |
|---|---|---|---|---|
| A | 10 | 0 | 0.43±0.02 | 336±22 |
| | 8 | 10 | 0.42±0.04 | 328±10 |
| C | 11 | 0 | 0.43±0.02 | 328±11 |
| | 8 | 12 | 0.40±0.03 | 348±3* |

* p<0.05, analysis of variance with Tukey's multiple range procedure

Body and organ weights

The organ and body weight changes are summarized in the following tables

Study A comparison of three different crude oils

| | Treatment group | | |
|---|---|---|---|
| Control | PBCO | SLCO | ALCO |
| Group size | | | |
| 10 | 8 | 6 | 7 |
| Change in body wt (g) | | | |
| +1.74±0.88 | -0.33±1.5 | -0.77±2.36* | -1.43±2.28* |
| Liver wt (g) | | | |
| 2.15±0.2 | 3.03±0.54* | 2.73±0.5 | 2.66±0.43* |

| | | | |
|---|---|---|---|
| Liver/body wt (g/g) | | | |
| 0.07±0 | 0.11±0.01* | 0.10±0.01* | 0.09±0.01* |
| Spleen/body wt (g/g) | | | |
| 3.52±0.61 | 2.46±0.24* | 2.49±0.29* | 2.17±0.66* |
| Thymus/body wt (g/g) | | | |
| 2.26±0.46 | 0.85±0.33* | 1.01±0.41* | 1.12±0.46* |

* P<0.05 Analysis of variance with Tukey's multiple range procedure

Study C: PBCO at 5 dose levels

| Body wt change (g) | Liver wt (g) | Organ/body wt (g/g) liver | spleen | thymus |
|---|---|---|---|---|
| Control group (10 mice) | | | | |
| +3.14±1.33 | 1.95±.18 | .06±.01 | 3.9±.49 | 2.7±.29 |
| 2 ml/kg/day (10 mice) | | | | |
| -0.88±2.57 | 2.32±.22 | .09±0 | 2.65±.62 | 1.61±.49* |
| 4 ml/kg/day (9 mice) | | | | |
| -0.86±1.24 | 2.38±.33* | .09±.01* | 2.57±.23* | 1.35±.32* |
| 8 ml/kg/day (8 mice) | | | | |
| -1.66±2.03* | 2.58±.48* | .1±.01* | 2.31±.67* | 1.03±.4* |
| 12 ml/kg/day (9 mice) | | | | |
| -1.66±1.71* | 2.69±.27* | .1±.01* | 2.2±.44* | .71±.22* |
| 16 ml/kg/day (7 mice) | | | | |
| -3.29±1.4* | 2.15±.35 | .1±.01* | 2.26±.87* | .65±.15* |
| Coefficient of determination ($R^2$) | | | | |
| 0.49*** | 0.08** | 0.48*** | 0.31*** | 0.64*** |

* P<0.05 Analysis of variance with Tukey's multiple range procedure
** P<0.03 Analysis of variance in regression
*** P<0.001 Analysis of variance in regression

Morphology

There were no gross abnormalities at necropsy except a reduction in the size of thymus glands in those animals that had received crude oil. Liver, kidney, spleen, lung and thymus from groups of 5 mice that had received 0, 5 or 10 ml PBCO for 5 days in the pilot study were examined histologically. The thymus glands of mice in the 10 ml/kg/day group had very thin cortices and reduced densities of lymphocytes in the remaining cortex compared to controls. Hepatocytes from these same animals had uniformly dense cytoplasm while control mouse hepatocytes had large areas of rarified cytoplasm typical of normal glycogen-replete mouse livers. The histology of the thymus glands of the 5 ml/kg/day group was intermediate between the controls and the 10 ml/kg/day group.
There were no histological lesions in the other tissues examined.
Overall, the results demonstrated minor hematological change, liver enlargement and atrophy of spleen and thymus.

**Test substance** : The following crude oils were examined:

Prudhoe Bay crude oil (PBCO) [a heavy crude]
South Louisiana crude oil (SLCO) [a light crude]
Arabian Light crude oil (ALCO)
In addition a bunker C oil was examined
A USP grade mineral oil and a corn oil were also included in the studies.

**Reliability** : (2) valid with restrictions
These studies were probably not carried out according to GLP and the studies did not include measurement of all the parameters normally measured in repeat dose studies. Although the results are of limited value they do nevertheless add some information on the effect of the repeated exposure of mice to crude oil.

(41)

## 5.5 GENETIC TOXICITY 'IN VITRO'

**Type** : Modified Ames Assay
**System of testing** : Ames test of a DMSO extract of test substance
**Test concentration** : 1 to 50µl/plate
**Metabolic activation** : With
**Year** : 1984
**GLP** : no data
**Test substance** : Beryl Crude (LIght crude oil)

**Method** : DMSO extracts were prepared by mixing 2 ml test material with 3 ml cyclohexane to homogeneity. 10 ml DMSO was then added and mixed thoroughly. The mixture was vortexed every 5 minutes for a total of 30 minutes. After 30 minutes the mixture was centrifuged and the DMSO layer (extract) removed and stored for testing.

The extract was only tested with metabolic activation in Salmonella typhimurium strain TA 98 at the following doses: 1, 3, 5, 7, 10, 15, 25 and 50 µl/plate. Additionally the DMSO extract of a carcinogenic oil was also tested in the same manner.
Positive control chemicals were: 2-aminoanthracene (2 µg)and benzoa(a)pyrene (5 µg).
The DMSO extract of a refrigerator oil was used as negative control (50 µl).
The metabolic activation mixture was derived from Araclor-induced hamster liver and this was use at eight times the standard concentration (0.4 ml rather than 0.05 ml).

The test material and control substances were added to tubes at the doses shown above and were incubated for 20 minutes with Salmonella broth culture. Colonies of histidine prototrophs were counted 48 hours after plate incubation.

Assay acceptance criteria

A linear dose response curve for mutagenicity must be obtained before the test material can be ranked for potency

Spontaneous and solvent control reversion rates for TA98 must fall between 20 and 50 revertants /plate

The slope of the dose response curve for the positive control carcinogenic oil must fall between 1.5 - 4.5 netrevertants/plate

Provided the above criteria are met the net revertants per plate is plotted

against dose and the best initial straight line response is determined by regression analysis. Test materials producing slopes less than 0.5 net revertants/µl are considered inactive.

**Result** : The No. revertants for each dose level of Beryl crude are as follows (NB data for the carcinogenic oil are not shown):

| Dose (µl) | No. of revertants |
|---|---|
| 0 (Spontaneous) | 43 |
| 0 (solvent control) | 39 |
| 50 | 113 |
| 25 | 85 |
| 15 | 74 |
| 10 | 67 |
| 7 | 61 |
| 5 | 57 |
| 3 | 47 |
| 1 | 48 |
| 2-AA control | 540 |
| BaP control | 366 |
| Refrigerator oil | 43 |

The slope for the Beryl crude was determined to be 2.5
The slope for the carcinogenic oil was 3.7

**Remark** : Similar studies were conducted for Arab light, MCSL, Lost Hills light and Belridge Heavy crudes.
The results were:

| Crude | Slope | Reference |
|---|---|---|
| Arab light | 3.8 | Mobil study 40965 |
| MCSL crude | 1.5 | Mobil study 40975 |
| Belridge Heavy | 1.7 | Mobil study 663850 |
| Lost Hills light | 0 | Mobil study 63838 |

**Conclusion** : A modified Ames assay conducted on five different crudes demonstrated that all but one (Lost Hills light) were mutagenic. The Lost Hills Light did not demonstrate any mutagenic activity.

**Reliability** : (2) valid with restrictions
It is not clear whether the studies were carried out according to GLP. Furthermore, the assay was designed as a screen for carcinogenic activity However, the results are useful to identify the mutagenic potential of crude oils.

(48) (49) (50) (61) (62)

**Type** : Cytogenetic assay
**System of testing** : Chinese Hamster Ovary cells
**Test concentration** : 1 to 20 µl DMSO extract/ml culture medium
**Metabolic activation** : With
**Result** : Negative
**Year** : 1991
**GLP** : no data
**Test substance** : Lost Hills Light crude

**Method** : Preparation of DMSO extract
10 ml DMSO was added to approximately 2 ml test substance.
The tube containing the mixture was vortexed every 5 minutes for one minute. After 30 minutes, the mixture was centrifuged and the DMSO layer

(Extract) was removed for testing.

Control substances:
Solvent control: DMSO
Negative control: Untreated flasks
Positive controls: Cyclophosphamide monohydrate at concentration of 10 µg/ml

Duplicate cultures were used - one set for metaphase analysis and the other for a determination of cytotoxicity (Mitotic Index). All cultures were conducted with metabolic activation.

CHO cells were exposed for two hours to test material at concentrations of 1, 2.5, 5, 10, 15 and 20 µl/ml. After two hours, treatment was terminated and the cultured cells were washed and re-fed with complete culture medium. Approximately 16 hours later, colcicine was added and two hours later the cells were harvested as described below.

Metaphase analysis
Metaphase cells were collected by tapping a flask to release the loosely attached mitotic cells into culture medium and pelleting them by centrifugation. The cells were resuspended and then fixed in methanol:acetic acid (3:1) and stored refrigerated until slide preparation. A minimum of 4 slides was made for each culture flask. 100 cells were examined microscopically per flask and were scored for structural chromosomal aberrations. Although gaps were recorded they were not used in the analysis for chromosomal aberration since their significance is questionable.

Cytotoxicity assay (Mitotic Index determination)
For this portion of the assay all cells in each flask were collected.
The medium in each flask was replaced and 5 ml of trypsin was added and after incubation for approximately 5 minutes the flasks were examined to ensure that the cells had rounded up. The cells were then released into the trypsin solution mechanically and the resulting suspension was centrifuged in the presence of medium. The centrifuged cells were swelled in hypotonic solution and were fixed in methanol:acetic acid as above. A minimum of 2 slides per flask were prepared for the determination of mitotic index. The slides were stained with Giemsa and at least 1000 nuclei per flask were scored as either mitotic or interphasic.

$$\text{The MI} = \frac{\text{No mitotic cells}}{\text{Total No of cells scored}} \times 100$$

Criteria for a positive or negative response
A test substance is considered to have elicited a positive response if at least one concentration shows a statistically significant increase in the proportion of cells with aberrations and a significant positive dose-response exists.
Biological significance of the result is also taken into account in determining the response.

**Result** : Determination of Mitotic Index (MI)
There was not a dose related decrease in MI at treatments that were not toxic. The MI at each of the dose concentrations of Lost Hills Light crude (LHL)was:

| Treatment | Mitotic Index |
|---|---|
| 20 µl/ml extract of LHL | no cells found |
| 15 µl/ml extract of LHL | no cells found |
| 10 µl/ml extract of LHL | 0.6 |
| 5 µl/ml extract of LHL | 10.7 |
| 2.5 µl/ml extract of LHL | 8.4 |
| 1 µl/ml extract of LHL | 12.2 |
| 20 µl/ml DMSO | 7.6 |
| Negative control | 10.4 |

Metaphase analysis
The concentrations analyzed for chromosomal aberrations were 5, 2.5 and 1.0 µg/ml.
There was no increase in the proportion of cells with structural chromosome aberrations (gaps excluded). Nor was a dose response observed. The data are:

| Treatment | No of cells with one or more aberrations (100 cells examined) |
|---|---|
| 5 µl/ml extract of LHL | 2 |
| 2.5 µl/ml extract of LHL2 | |
| 1 µl/ml extract of LHL | 1 |
| DMSO (solvent control | 2 |
| Negative control | 2 |
| CP (positive control) | 28 |

**Reliability** : (1) valid without restriction
Despite the fact that whether the study was carried out to GLP is not clear, it is, nevertheless, reliable and valid.

(70)

**Type** : Cytogenetic assay
**System of testing** : Chinese Hamster Ovary cells
**Test concentration** : 1 to 20 µl DMSO extract/ml culture medium
**Metabolic activation** : With
**Result** : Negative
**Year** : 1991
**GLP** : no data
**Test substance** : Belridge Heavy crude

**Method** : Preparation of DMSO extract
10 ml DMSO was added to approximately 2 ml test substance. The tube containing the mixture was vortexed every 5 minutes for one minute. After 30 minutes, the mixture was centrifuged and the DMSO layer (Extract) was removed for testing.

Control substances:
Solvent control: DMSO

Negative control: Untreated flasks
Positive controls: Cyclophosphamide monohydrate at concentration of 10 µg/ml

Duplicate cultures were used - one set for metaphase analysis and the other for a determination of cytotoxicity (Mitotic Index). All cultures were conducted with metabolic activation.

CHO cells were exposed for two hours to test material at concentrations of 1, 2.5, 5, 10, 15 and 20 µl/ml. After two hours, treatment was terminated and the cultured cells were washed and re-fed with complete culture medium. Approximately 16 hours later, colcicine was added and two hours later the cells were harvested as described below.

Metaphase analysis
Metaphase cells were collected by tapping a flask to release the loosely attached mitotic cells into culture medium and pelleting them by centrifugation. The cells were resuspended and then fixed in methanol:acetic acid (3:1) and stored refrigerated until slide preparation. A minimum of 4 slides was made for each culture flask. 100 cells were examined microscopically per flask and were scored for structural chromosomal aberrations. Although gaps were recorded they were not used in the analysis for chromosomal aberration since their significance is questionable.

Cytotoxicity assay (Mitotic Index determination)
For this portion of the assay all cells in each flask were collected. The medium in each flask was replaced and 5 ml of trypsin was added and after incubation for approximately 5 minutes the flasks were examined to ensure that the cells had rounded up. The cells were then released into the trypsin solution mechanically and the resulting suspension was centrifuged in the presence of medium. The centrifuged cells were swelled in hypotonic solution and were fixed in methanol:acetic acid as above. A minimum of 2 slides per flask were prepared for the determination of mitotic index. The slides were stained with Giemsa and at least 1000 nuclei per flask were scored as either mitotic or interphasic.

$$\text{The MI} = \frac{\text{No mitotic cells}}{\text{Total No of cells scored}} \times 100$$

Criteria for a positive or negative response
A test substance is considered to have elicited a positive response if at least one concentration shows a statistically significant increase in the proportion of cells with aberrations and a significant positive dose-response exists. Biological significance of the result is also taken into account in determining the result.

**Result** **:** Determination of Mitotic Index (MI)
There was not a dose related decrease in MI at treatments that were not toxic. The MI at each of the dose concentrations of Belridge Heavy crude (BH)was:

| **Treatment** | **Mitotic Index** |
|---|---|
| 20 µl/ml extract of BH | no cells found |
| 15 µl/ml extract of BH | no cells found |
| 10 µl/ml extract of BH | no cells found |
| 5 µl/ml extract of BH | 11.2 |
| 2.5 µl/ml extract of BH | 10.2 |

| | |
|---|---|
| 1 µl/ml extract of BH | 13.1 |
| 20 µl/ml DMSO | 7.6 |
| Negative control | 10.4 |

Metaphase analysis
The concentrations analyzed for chromosomal aberrations were 5, 2.5 and 1.0 µg/ml. There was no increase in the proportion of cells with structural chromosome aberrations (gaps excluded). Nor was a dose response observed. The data are:

| **Treatment** | **No of cells with one or more aberrations (100 cells examined)** |
|---|---|
| 5 µl/ml extract of LHL | 1 |
| 2.5 µl/ml extract of LHL | 2 |
| 1 µl/ml extract of LHL | 1 |
| DMSO (solvent control | 2 |
| Negative control | 2 |
| CP (positive control) | 28 |

**Reliability** : (1) valid without restriction
Despite the fact that whether the study was carried out to GLP is not clear, it is, nevertheless, reliable and valid.

(69)

**Type** : Ames test
**Metabolic activation** : with and without
**Year** : 1981
**GLP** : no data
**Test substance** : South Louisiana crude (Light crude oil)

**Method** : The authors summarized the protocol in the following table:

Test strains
Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100

Liver homogenate
S-9 from male Charles River CD rat liver 500 mg Aroclor/kg for 5 days

Procedure
Treatment without activation: 2 ml top agar, 0.1 ml dissolved test chemical, 0.1 ml bacterial culture ($10^8$ cells)
Treatment with activation: 2 ml top agar, 0.1 ml dissolved test chemical, 0.1 ml bacterial culture ($10^8$ cells) plus 0.5 ml S-9 mix contains per milliliter 0.3 ml S-9 (1 g tissue + 3 ml 0.15M KCl), 8 mM Mg $Cl_2$, 33 mM KCl, 5mM glucose-6-phosphate, 4 mM NADP, and 100 mM sodium phosphate (pH 7.4).
Incubation period: 48 hr

Design: Preliminary toxicity determination
Duplicate plates/point
Five test concentrations and solvent and positive controls
Duplicate experiments

Solvent Dimethyl sulfoxide

Data analysis
Mutagenic: statistically significant. Increase in total revertant colony number (P≤ 0.01) and dose response (P≤ 0.01)

Scoring

| | Revertant/µg | Induced frequency/ Spontaneous frequency |
|---|---|---|
| (-) | <0.01 | -* |
| (+) | 0.1 to 0.01 | <5 |
| (++) | >0.1 | >5 |

* No greater than response

**Result** : The results are given in the following table

| Sample | Mutagenic activity (with activation) | | | |
|---|---|---|---|---|
| | TA-1537 | TA-1538 | TA-98 | TA-100 |
| Benzo[a]pyrene | ++ | ++ | ++ | ++ |
| South Louisiana crude | - | - | - | + |

It is not clear from the report whether any activity was detected in the assay without metabolic activation.

**Reliability** : (4) not assignable
The study is not fully reported (data for the assay without metabolic activation are not given)

(9)

**Type** : Ames test
**Metabolic activation** : with and without
**Result** : Negative
**Year** : 1982
**GLP** : no data
**Test substance** : Wilmington crude

**Method** : Salmonella typhimurium strains TA 98 and TA 100 were used in this study. The test sample was suspended in Tween 80 prior to testing. Each sample was weighed in a glass vial and then an equal weight of Tween 80 was added. The oil and detergent were stirred until thoroughly homogenized. Distilled water was then added dropwise with continuous stirring until a stable, homogenous suspension was obtained. The concentration of the mixture was then adjusted to 10% sample - 10% Tween 80 (w/v) by the addition of more distilled water.
The assay was carried out in triplicate with and without metabolic activation by S9 liver homogenate that was prepared from Aroclor-induced Sprague-Dawley rats. The S9 homogenate contained 13 mg of protein/ml and was stored at -70°C. S9 homogenate was used at a concentration of 10% (v/v) in the assay.
BaP was used as the positive control.
Negative control plates for determination of the spontaneous reversion rate were treated with 400µl or less of 10% Tween 80, a non-toxic and non-mutagenic dose.

No other experimental details are given in the paper, but reference is made to the papers by Ames that describe the methodology.

**Result** : Addition of up to 400µl Tween 80 per plate did not alter the spontaneous

reversion rates of either TA 98 or TA 100. Tween 80 had no effect on the mutagenicity of BaP, which induced similar numbers of revertants in TA 98 with S9 activation whether dissolved in DMSO or Tween 80.
The crude oil sample was inactive in all bacterial mutagenicity tests.

**Reliability** : (4) not assignable
The numbers of revertants were not given. The result was simply stated by the authors.

(43)

**Type** : Sister chromatid exchange assay
**System of testing** : Human lymphocytes
**Year** : 1982
**GLP** : no data
**Test substance** : Wilmington crude oil

**Method** : Heparinized blood was obtained from a healthy 25-year old male. [The method for culture of the lymphocytes was not described but reference was given which describes the method].
Benzo(a)pyrene (BaP) and N-methyl-N'-nitro-N-nitroso guanidine (MNNG) were used as positive control substances.

The test sample was suspended in Tween 80 prior to testing.
Each sample was weighed in a glass vial and then an equal weight of Tween 80 was added. The oil and detergent were stirred until thoroughly homogenized. Distilled water was then added dropwise with continuous stirring until a stable, homogenous suspension was obtained. The concentration of the mixture was then adjusted to 1% sample - 1% Tween 80 (w/v) by the addition of more distilled water. Lymphocyte cultures were treated 18 hours after initiation by
(a) continuous exposure to test chemical without exogenous activation or
(b) by exposure for 2.5 hours with activation by S9.
[procedure described elsewhere by Stetka and Wolff (1976) for metabolic activation].
In tests with S9, cells were collected by centrifugation at 150 x g for 10 minutes, resuspended in 5 ml of McCoy's 5A medium containing only antibiotics and treated with microliter quantities of test material or solvent. An aliquot of 0.25 ml of S9 mix containing 10% S9 (v/v) was added to each culture. Cells were incubated for 2.5 hours with occasional mixing. The cells were collected, washed once in medium with 5% fetal bovine serum and resuspended in 8 ml of culture medium containing BrdUrd for further incubation. In experiments testing MNNG, lymphocytes that had been cultured for 18 hours were suspended in medium without serum, S9 or BrdUrd, treated for 2 hours with MNNG in acetone or acetone alone (controls), washed as above, resuspended in complete medium with BrdUrd and incubated further. At 68 hours after the cultures were initiated, Colcemid (0.01µg/ml) was added; 4 hours later, the cells were harvested by centrifugation, subjected to hypotonic shock for 7 minutes in 0.075M KCl, fixed in 3 changes of cold methanol-acetic acid (3:1) and spread on chilled, wet microslides that had been pre-cleaned in 95% ethanol. The preparations were stained by the Hoechst 33258-black light-Giemsa method and numbers of SCE were counted in 25 metaphase cells containing 40-48 chromosomes

**Result** : Tween 80 was not cytotoxic and did not induce increases in the numbers of SCE in cultures of lymphocytes treated with 100 ppm Tween 80 for 54 hours without activation or for 2.5 hours with S9 activation.
MNNG, a strong alkylating agent, induced a greater than 5-fold increase in

SCE at a concentration of only 0.15 ppm.
The response to BaP in the lymphocyte cultures indicated that the S9 metabolic system was functional. Although increases in SCE of only 50% over the number in control cultures were induced, these increases are statistically significant (P<0.001). In the presence of S9 mix, only 1/16 the amount of BaP was required to induce the same number of SCE observed without S9, indicating that metabolism had occurred. However, 0.5µg/ml of BaP with S9 mix was toxic to these cultures, as indicated by poor cell morphology and a reduced number of cells in mitosis.
Wilmington crude did not induce an increase in SCE at any dose concentration with or without S9 activation.
The data are summarized in the following table.

| Treatment | Dose (ppm) | No. of cells | SCE/cell ±S.E. |
|---|---|---|---|
| 24 hours exposure, without S9 | | | |
| Tween 80 | 0 | 25 | 8.4±0.5 |
| | 100 | 25 | 9.7±0.6 |
| B(a)P | 0 | 25 | 7.7±0.6 |
| | 4 | 25 | 10.8±1.2 |
| | 8 | 25 | 15.1±0.9** |
| Wilmington crude oil | | | |
| | 40 | 25 | 10.7±1.2 |
| | 50 | 25 | 9.9±0.8 |
| 2.5 hours exposure, with S9 | | | |
| Tween 80 | 40 | 25 | 9.6±0.0 |
| | 100 | 25 | 8.0±0.5 |
| B(a)P | 0 | 25 | 10.9±1.9 |
| | 0.2 | 25 | 13.4±0.8** |
| | 0.5 | 10 | 15.9±1.2** |
| | 1.0 | 0 | Toxic |
| MNNG | 0 | 25 | 8.7±0.4 |
| | 0.015 | 23 | 11.7±0.5** |
| | 0.05 | 23 | 28.3±1.3** |
| | 0.15 | 21 | 48.5±2.2** |
| Wilmington crude oil | | | |
| | 20 | 25 | 8.8±0.7 |
| | 30 | 25 | 6.6±0.5 |

** P<0.001

**Reliability** : (2) valid with restrictions
Complete description of study not given in publication. Some methods details published elsewhere

(43)

## 5.6 GENETIC TOXICITY 'IN VIVO'

**Type** : Micronucleus assay
**Species** : Rat
**Sex** : male/female
**Strain** : Sprague-Dawley
**Route of admin.** : Dermal
**Exposure period** : 13 weeks
**Doses** : 30, 125 and 500 mg/kg/day
**Result** : Negative
**Year** : 1990
**GLP** : Yes
**Test substance** : Lost Hills Light crude

**Method** : The animals used in this study were taken from a 13 week repeated dermal administration study (Study 63834).
Bone marrow was removed from the femurs of five male and five female rats from each dose group (0, 30, 125 and 500 mg/kg/day).
A nearly pure erythrocyte fraction was obtained and two slides prepared for each animal. 1000 PCEs (polychromatic erythrocyte) and 1000 NCEs (normochromatic erythrocyte) were scored to determine the percentage of micronucleated erythrocytes. 1000 erythrocytes were scored to determine the ratio of PCEs to NCEs.

**Result** : The results are given in the following table. Standard deviations have not been included in the table. However, statistical analyses were conducted and it was found that there were no significant differences between treated and control animals.
It was concluded that the test material was not cytotoxic to red blood cell formation and furthermore did not increase the formation of micronucleated PCEs or NCEs in the bone marrow.

| Dose mg/kg | Sex | No of animals | PCE/NCE | MNPCEs (%) | MNNCEs (%) |
|---|---|---|---|---|---|
| 0 | F | 5 | 1.34 | 0.1 | 0 |
| 0 | M | 5 | 0.79 | 0.02 | 0 |
| 0 | M+F | 10 | 1.06 | 0.06 | 0 |
| 30 | F | 5 | 1.26 | 0.04 | 0 |
| 30 | M | 5 | 0.68 | 0 | 0.02 |
| 30 | M+F | 10 | 0.97 | 0.02 | 0.01 |
| 125 | F | 5 | 1.18 | 0 | 0 |
| 125 | M | 5 | 1.17 | 0.06 | 0 |
| 125 | M+F | 10 | 1.17 | 0.03 | 0 |
| 500 | F | 5 | 1.25 | 0.02 | 0 |
| 500 | M | 5 | 0.83 | 0.04 | 0 |
| 500 | M+F | 10 | 1.04 | 0.03 | 0 |

PCE Polychromatic erythrocytes
NCE Normochromatic erythrocytes
%MNPCEs % Micronucleated PCEs
%MNNCEs % Micronucleated normochromatic erythrocytes

**Remark** : Two further studies have been reported.

1. Belridge heavy crude.
   The results of this study were the same as those for the study on Lost Hills Light described above. (Mobil study 63847)

2. Wilmington crude
   Results in ICR Swiss mice showed that Wilmington crude oil had relatively little capacity to cause chromosome breakage and/or non-disjunction. (Lockard et al, 1982)

**Reliability** : (1) valid without restriction
(43) (64) (71) (72)

**Type** : Sister chromatid exchange assay
**Species** : Mouse
**Sex** : Male
**Strain** : ICR
**Route of admin.** : i.p.
**Doses** : 1.8, 3.6 &7.2 g/kg
**Year** : 1982
**GLP** : no data
**Test substance** : Wilmington crude

**Method** : The assay was carried out in groups of 3 male 10-14 week old Sch:ICR mice.
The mice were lightly anesthetized with ether and a 50 mg tablet of BrdUrd was inserted beneath the skin on the dorsal side of the neck of each animal. After 1 hour, treatment was given by a single i.p. injection at doses of 1.8, 3.6 and 7.2 g/kg (equivalent to 0.8, 0.4 and 0.2 times the approximate LD50).
Control groups of mice were injected with B(a)P at doses of 0.16, 0.08 and 0.04 g/kg, Cyclophosphamide at doses of 0.01 and 0.005 g/kg in distilled water. Trioctanoin and distilled water were also given to negative solvent controls.
22 hours after treatment, animals were injected i.p. with Colchicin (10 mg/kg). 2 hours later the mice were sacrificed by cervical dislocation and their femurs were removed. The bone marrow cells were flushed out with saline. Pooled cells from the 2 femurs of each animal were treated with hypotonic KCl for 30 minutes, were fixed and were spread on slides. The slides were stained and the numbers of SCE counted in 25 metaphase cells from each animal, each metaphase having 36-42 chromosomes.

**Result** : Control animals injected with the solvent trioctanoin had a mean of 5.3 SCE/cell. A slight but significant increase in SCE was found in animals treated with the highest dose only of Wilmington crude oil, but the response was not dose related.
B(a)P and cyclophosphamide induced dose-related increases in SCE.
The actual results are shown in the following table.

| Treatment | Dose | No. survivors/ No. treated | No. cells examined | SCE/ cell±S.E. |
|---|---|---|---|---|
| Trioctanoin | 0.5 ml/ mouse | 7/8 | 175 | 5.3±0.4 |
| B(a)P | 0.16 g/kg | 3/3 | 75 | 9.7±0.6*** |
| | 0.08 | 3/3 | 75 | 8.5±0.5*** |
| | 0.04 | 3/3 | 75 | 7.8±0.5*** |
| Cyclophosphamide | | | | |
| | 0.01 g/kg | 3/3 | 75 | 19.5±0.6*** |
| | 0.005 | 3/3 | 75 | 14.6±0.6*** |
| Wilmington crude oil | | | | |
| | 7.2 g/kg | 3/3 | 75 | 6.6±0.4** |
| | 3.6 | 3/3 | 50 | 5.0±0.3 |
| | 1.8 | 3/3 | 75 | 6.4±0.4 |

** P<0.05
*** P<0.001

**Reliability** : (2) valid with restrictions
Probably not to GLP and method description is not comprehensive.

(43)

## 5.7 CARCINOGENICITY

**Species** : Mouse
**Sex** : Male
**Strain** : C3H
**Route of admin.** : Dermal
**Exposure period** : 18 months
**Frequency of treatm.** : Twice weekly
**Doses** : 50 mg per application
**Result** : Positive
**Control group** : Yes
**GLP** : no data
**Test substance** : Two crudes C & D

**Method** : 50 mg undiluted test material was applied to the shorn interscapular region of groups of 50 male mice. Application was twice weekly for 18 months or until grossly-observable cancer was found. Each animal was observed weekly for the appearance of tumors. The percentage of animals developing tumors and the time to appearance of first tumor were recorded. No distinction was made between histologically benign or malignant lesions.

**Result** : The % tumors and average latencies for the two crudes C & D are shown below.

| | % Tumors* | Latency** |
|---|---|---|
| Crude C | 33 | 76 |
| Crude D | 56 | 64 |

* Based on Final Effective Number ** Average latency in weeks

**Test substance** : Two crudes were included in this study. They were characterized as follows:

| | Crude C | Crude D |
|---|---|---|
| Sulfur (wt%) | 0.21 | 2.54 |
| Distillation yield (vol%) | | |
| Int-120°F | 0.0 | 3.3 |
| 120-350 | 2.5 | 18.3 |
| 350-550 | 41.9 | 19.5 |
| 550-700 | 21.0 | 12.2 |
| 700-1070 | 31.2 | 26.8 |
| 1070 btms | 3.4 | 19.9 |
| | | |
| Composition of 550-700°F | | |
| Fraction (wt%) | 5.1 | 33.3 |
| Monocycloparaffins | 8.1 | 11.5 |
| Polycycloparaffins | 46.6 | 19.5 |
| Total saturated hydrocarbons | 59.8 | 64.3 |
| Mononuclear aromatics | 21.5 | 11.0 |
| Di- and Trinuclear aromatics | 14.4 | 21.8 |
| Polynuclear aromatics | 1.0 | 1.8 |
| Total aromatic hydrocarbons | 36.9 | 34.6 |
| Resins | 3.3 | 1.1 |
| Asphaltenes | 0.0 | 0.0 |

**Reliability** : (2) valid with restrictions

(42)

**Species** : Mouse
**Sex** : male/female
**Strain** : C3H
**Route of admin.** : Dermal
**Exposure period** : 105 weeks
**Frequency of treatm.** : Three times weekly
**Doses** : 25 mg per application
**Control group** : Yes
**Year** : 1988
**GLP** : no data
**Test substance** : Heavy crude oil, San Joaquin Valley

**Method** : The study reported by Clarke et al was a comparison of the carcinogenicity of shale and petroleum crude oil and of several distillate streams derived from the two crude oils. Only the details relating to petroleum crude and the respective positive and negative controls are included in this summary. The crude oil, positive and negative control materials were applied three times weekly at a dose of 25 mg/application to 25 male and 25 female mice. The materials were applied to the shorn dorsal thoracic region which was shaved as necessary during the study. Dosing was continued for up to 105 weeks.
Animals were observed twice daily for overt signs of toxicity, moribundity and mortality. Animals were weighed weekly for the first 13 weeks and every two weeks thereafter. Animals were palpated weekly for external and internal masses.

Every animal was subjected to a complete necropsy. All organs and the remainder of the carcass were fixed and stored. Body weights and weights of liver, kidneys, brain and gonads were recorded. A microscopic examination was made of histological slides prepared from skin from a

control site, skin from treated site, kidney, liver, lungs, gonads, urinary bladder, spleen, sternal bone marrow and any other organ considered abnormal at necropsy.

**Result** : Survival was affected by treatment with petroleum crude oil.
After one year, survival was approximately 70% and by week 70 survival was approximately 50%. There were few survivors at 105 weeks. This is in contrast to the negative control group with 50% survival at the end of the study. Dermal irritation at the test site first appeared at 271 days and males developed irritation earlier than the females [no details given]. Necrosis occurred and was characterized as loss of integrity of the skin with visible cracking, separation and sloughing of skin often revealing underlying mesenchymal tissue.

There was no indication of toxic or oncogenic effects on internal organs.

The authors reported the occurrence of reactive hyperplasia in the spleen and bone marrrow and this was characterized by increased populations of lymphocytes, plasma cells and neutrophils or granulocyte precursors and was attributed to the inflammation, necrosis and other tissue alterations occurring at the test site. [There is no indication in the publication whether this finding occurred in the group treated with petroleum crude oil.]

The tumor incidences that were recorded are shown in the following table:

| | **Crude oil** | **B(a)P** | **Control** |
|---|---|---|---|
| Incidence (%) | 37/44 (84) | 49/49 | 0/46 |
| Latency (weeks) | 62±13 | 28±4 | |
| Squamous cell carcinoma | 29 (66) | 49 (100) | 0 |
| Fibrosarcoma | 7 (16) | 0 | 0 |

**Test substance** : The San Joaquin Valley crude oil is a heavy crude with the following characteristics:

| | |
|---|---|
| Gravity (°API) | 10.7 |
| Boiling range (°F) | 410-1071 |
| Nitrogen (ppm wt) | 8150 |
| Sulfur (ppm wt) | 10,510 |

Due to its high viscosity, the crude oil was administered as a 2:1 dilution in mineral oil.
Mineral oil USP was used as a negative control
B(a)P (0.15% w/v in mineral oil) was used as a positive control

**Reliability** : (2) valid with restrictions
Few experimental details are given in this publication. Despite this, the information may be used in the overall assessment of the carcinogenic potential of crude oil

(11)

### 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Gavage |
| **Frequency of treatm.** | : | Various |
| **Year** | : | 1987 |
| **GLP** | : | no data |
| **Test substance** | : | Prudhoe Bay crude oil (Heavy crude oil) |

**Method** :

Presumed pregnant female Sprague-Dawley rats were used for this series of studies.

Prudhoe Bay crude oil was administered by gavage according to the following schedules

1. As a single dose (5 ml/kg) on either day 3, 6, 11, 15 or 17 of gestation.
2. Daily from gestation days 6-17 incl at a dose level of 1.0 or 2.0 ml/kg.
3. As a single dose on day six of gestation at either 2, 5, 7 or 10 ml/kg.

Respective controls received equivalent amounts of saline.

On day 18 of pregnancy, animals were sacrificed and the numbers and position of implantations, resorptions and dead fetuses were recorded. The live fetuses were removed, weighed and inspected for gross external abnormalities with the aid of a dissection microscope. Skeletal examinations and examination of soft internal tissues were not carried out in this preliminary study.
Student's t-test was used for comparing control and experimental results. P values were derived from a 2-tailed table of Student's values for t. The level of significance chosen was $P<0.05$.

**Result** :

There were no maternal deaths following oral administration of PBCO although at high doses the animals exhibited signs of intoxication (crouching, indicative of acute irritation).
Maternal body weight changes were significantly less in animals that had been treated with PBCO at early days of gestation, in particular those animals given PBCO on day 6 of gestation only.
The body weight data are shown in the following tables. Note that although the author provided data on mean and standard deviation, for simplicity this summary only contains mean values (SDs are not shown). Statistically significant differences are indicated.
Body wt changes for dams given a single (5 ml/kg) dose of PBCO. Figures in parenthesis indicate group size

| **Treatment** | **Body weight change (g on days indicated)** | | | | |
|---|---|---|---|---|---|
| **Day** | **0-6** | **6-11** | **11-15** | **15-18** | **0-18** |
| Control (11) | 30.4 | 32.6 | 28 | 30 | 121 |
| 3 (9) | 32.75 | 28.25 | 23.5* | 31 | 115.22 |
| 6 (10) | 28.8 | 22.4* | 24.2* | 27.8 | 103.2* |
| 11 (10) | 34.8 | 35.6 | 16* | 35.2 | 122.6 |
| 15 (10) | 32.5 | 33.5 | 28 | 35.3 | 131.5 |
| 17 (10) | 31.67 | 34 | 27.33 | 31.67 | 124.67 |

* Significant from control ($P<0.05$)

Effect of different doses of PBCO on dam body weight changes
No. of animals shown in parenthesis

| Treatment | Body weight change (g on days indicated) | | | | |
|---|---|---|---|---|---|
| | 0-6 | 6-11 | 11-15 | 15-18 | 0-18 |
| Control (8) | 27.33 | 25.67 | 27.33 | 32 | 112.33 |
| Dose (ml/kg) given on day 6 only | | | | | |
| 2 (8) | 27.5 | 25 | 23* | 27.5 | 1.3 |
| 5 (10) | 26.21 | 18.08* | 19.5* | 26.92* | 91.22* |
| 7 (8) | 26.67 | 17.33* | 15.35* | 26.92* | 87.67* |
| 10 (8) | 30.67 | 14.33* | 11* | 20* | 75.67* |
| Dose given on days 6-17 daily | | | | | |
| 1 (10) | 31 | 21* | 23.5* | 22* | 97.5* |
| 2 (10) | 27.5 | 16.5 | 13* | 17* | 75* |

* Significant from control (P<0.05)

The author concluded that the reduced body weight gains were probably due to the high resorption rates that occurred.

Effects on fetal viability and development
The results of the effect of administering PBCO as a single dose of 5 ml/kg at different stages of gestation are summarized below:

Values shown are mean±SD
* significant from control (p<0.05)

| No. implants per dam | Resorptions/ dead fetuses (%) | No. live fetuses | Fetal weight (g) | Fetal crown-rump length (mm) |
|---|---|---|---|---|
| Day 0 | | | | |
| 13.5±2.39 | 4.68±.93 | 13±1.24 | 1.267±.09 | 22.2±1.44 |
| Day 3 | | | | |
| 14.5±1.92 | 10.77±1.02* | 11.25±1.5 | 1.19±.08* | 20±1.73* |
| Day 6 | | | | |
| 3.23±1.43 | 15.48±1.32* | 11.02±1.49* | 1.2±.09* | 1.16±1.38 |
| Day 11 | | | | |
| 2.9±1.26 | 8.22±1.08* | 11.4±.89* | 1.287±.11 | 21.56±1.37 |
| Day 15 | | | | |
| 12.72±1.63 | 5.02±1.19 | 12.5±.71 | 1.313±.083 | 22.65±1.33 |
| Day 17 | | | | |
| 13±1.8 | 3.62±.89 | 12.33±1.15 | 1.304±.124 | 23.01±1.41 |

These results demonstrate that the incidence of resorptions and dead fetuses was increased in the animals given PBCO during the early days of pregnancy whereas those given PBCO during the later stages of pregnancy were unaffected. The greatest effect was on day 6 of gestation.

Repeated daily administration of low doses (1 or 2 ml/kg) on days 6 through 7 showed an additive effect and at a dose level of 2 ml/kg/day resulted in up to 40% fetal deaths. These data are shown in the following table.

The effect of different doses of PBCO

Values shown are mean ± SD
* significant from control (p<0.05)

| No. implants per dam | Resorptions/ dead fetuses (%) | No. live fetuses | Fetal weight (g) | Fetal crown-rump length (mm) |
|---|---|---|---|---|
| Dose (ml/kg) | | | | |
| 0 ml/kg | | | | |
| 13.95±2.23 | 3.96±0.87 | 13.72±1.56 | .309±.091 | 21.62±1.17 |
| Day 6 | | | | |
| 2 ml/kg | | | | |
| 14±2.63 | 6.84±1.21 | 13.85±2.33 | 1.326±.119 | 21.78±1.54 |
| 5 ml/kg | | | | |
| 13.26±1.95 | 12.67±1.18* | 11.73±1.47* | 1.216±.09* | 21.54±1.27 |
| 7 ml/kg | | | | |
| 12.93±2.35 | 13.23±1.35* | 11.68±1.46* | 1.2±.109* | 21.28±.89 |
| 10 ml/kg | | | | |
| 14.12±2.12 | 22.86±1.98* | 10.5±1.59* | 1.192±.108* | 0.73±1.19 |
| Day 6-17 (daily) | | | | |
| 1 ml/kg | | | | |
| 13.92±2.46 | 17.5±2.02* | 10±1.95* | 1.184±.107 | 0.16±1.34* |
| 2 ml/kg | | | | |
| 12.93±1.98 | 43.8±1.92* | 8.72±1.34* | 1.182±.097* | .96±1.31* |

In conclusion, administration of PBCO to pregnant females resulted in an increased incidence of resorptions, increased fetal death and decreased fetal body weight. These effects occurred at doses which were maternally toxic.

**Reliability** : (2) valid with restrictions
Probably not conducted to GLP. Study was a preliminary study and was limited in scope. Only information on embryo and fetotoxicity was derived in this study.

(40)

**Species** : Rat
**Sex** : Female
**Strain** : Sprague-Dawley
**Route of admin.** : Dermal
**Frequency of treatm.** : Daily
**Duration of test** : Days 0-19 of gestation
**Doses** : 30, 125 and 500 mg/kg/day
**Control group** : yes, concurrent no treatment
**Year** : 1991
**GLP** : no data
**Test substance** : Belridge Heavy Crude

**Method** : Groups of twelve presumed-pregnant Sprague-Dawley rats were distributed into six groups as shown below. The test material was applied to the shorn dorsal skin once daily at the doses shown from day 0 to day 19 of gestation. The animals were shaved weekly and were fitted with collars to prevent ingestion of the test material.

| Group | Dose level |
|---|---|
| Prenatal groups | |
| 1 | Sham control |
| 2 | 30 mg/kg/day |
| 3 | 125 mg/kg/day |
| 4 | 500 mg/kg/day |
| Postnatal groups | |
| 5 | Sham control |
| 6 | 500 mg/kg/day |

Animals were observed at least once daily throughout gestation until sacrifice for signs of pathosis, abortion, premature delivery, dystocia and/or death. Dams and their litters were observed postpartum days 0 through 4. On day 0 postpartum, pups were examined for external malformations and variations and were observed daily for the presence of milk in their stomachs.
Body weights and food consumption of all prenatal group females were recorded at regular intervals throughout gestation and for the post natal groups throughout gestation and body weights only on postpartum days 0 and 4.
On day 20 of gestation all prenatal group animals were sacrificed. The abdominal cavity was exposed and the reproductive organs examined. Following removal of the uterus and ovaries the remains were subjected to macroscopic examination and the liver and thymus were weighed.
The uterus and ovaries of each animal were examined grossly. The no. of corpora lutea per ovary was recorded. Ovaries of non pregnant females were examined and then discarded. The uterine contents of each pregnant animal were exposed and the number and location of all implantations were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation. Blood samples were collected at the time of sacrifice and these were used for a range of serum chemistry determinations.
Each live fetus was weighed and its sex determined and examined for external anomalies. After gross examination the fetuses were equally distributed into two groups. One group was processed for an evaluation of visceral anomalies and the other group for skeletal anomalies.
Females in the postnatal groups with surviving offspring were sacrificed on post partum day 4 and the abdominal cavity was exposed for a gross examination of the reproductive organs. The uterus was also examined and then discarded. All organs were examied macroscopically and the thymus and liver were weighed.

**Remark** : This study was also reported in the open literature by Feuston et al. (29)

**Result** : Clinical observations

Treatment related clinical observations in the prenatal and postnatal groups consisted of skin irritation in the 500 mg/kg/day group. This included erythema, edema, scabs and open sores at the treatment site. There was also a red vaginal discharge in animals in this group. One high dose prenatal female had excessive vaginal discharge and, after being found moribund, was sacrificed on gestation day 14. Uterine examination revealed that all but two fetuses had been resorbed.
Body weights and food intakes of the 500 mg/kg/day prenatal groups were reduced compared to controls (reduced by approx 31%). Other dose groups were unaffected.

Necropsy findings

With the exception of an approximately 11% increase in relative liver

weight in the 500 mg/kg/day group, no other organ or relative organ weight changes were recorded. [The authors comment that the observed increase in relative liver weight was possibly attributable to the decreased body weight of this group].
A prominent vascular pattern of the liver was observed in one female of the 125 mg/kg/day group and two animals in the highest dose group.

Serum chemistry
The only observation was a 38% reduction of total bilirubin in the prenatal 500 mg/kg/day group.

Reproductive and fetal evaluations
Effects only occurred in the highest dose group (500 mg/kg/day). These included

an increase in the mean number/percent resorptions (5.3 compared to 1.3 and 35% compared to 7.8% respectively) and a corresponding decrease in litter size (10.8 compared to 14.5).

A decrease in mean fetal body weights for all viable fetuses (3.4 compared to 3.7).

Male fetuses in this dose group seemed to be more affected than females.
Fetal skeletal anomalies recorded were limited to incomplete ossification of the nasal bones and caudal centra.
There were no treatment related visceral anomalies.

Post partum observations
During the post partum period there were no clinical observations of note except some persistent skin irritation. There were no findings at necropsy.

Litter data
Two females in the 500 mg/kg/day group had no viable offspring; their litters were totally resorbed. The incidence of pup mortality was two times greater in the group treated with crude oil during the lactation period. Mean duration of gestation was unaffected, but it was noted that the majority of treated females delivered either in the afternoon of day 22 or morning of day 23 whereas the controls delivered in the morning of day 22. [The biological significance of this observation is uncertain.]

**Reliability** : (1) valid without restriction

(29) (67)

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Dermal |
| **Frequency of treatm.** | : | Daily |
| **Duration of test** | : | Days 0-19 of gestation |
| **Doses** | : | 125, 500 and 2000 mg/kg/day |
| **Control group** | : | yes, concurrent no treatment |
| **NOAEL maternal tox.** | : | 125 mg/kg bw |
| **NOAEL teratogen.** | : | 500 mg/kg bw |
| **Year** | : | 1991 |
| **GLP** | : | no data |
| **Test substance** | : | Lost Hills Light |

**Method** : Groups of twelve presumed-pregnant Sprague-Dawley rats were distributed into six groups as shown below. The test material was applied

to the shorn dorsal skin once daily at the doses shown from day 0 to day 19 of gestation. The animals were shaved weekly and were fitted with collars to prevent ingestion of the test material.

| Group | Dose level |
|---|---|
| Prenatal groups | |
| 1 | Sham control |
| 2 | 125 mg/kg/day |
| 3 | 500 mg/kg/day |
| 4 | 2000 mg/kg/day |
| Postnatal groups | |
| 5 | Sham control |
| 6 | 20000 mg/kg/day |

Animals were observed at least once daily throughout gestation until sacrifice for signs of pathosis, abortion, premature delivery, dystocia and/or death. Dams and their litters were observed postpartum days 0 through 4. On day 0 postpartum, pups were examined for external malformations and variations and were observed daily for the presence of milk in their stomachs.
Body weights and food consumption of all prenatal group females were recorded at regular intervals throughout gestation. For the post natal groups body weights and food intakes were recorded throughout gestation but only body weights were recorded on postpartum days 0 and 4.
On day 20 of gestation all prenatal group animals were sacrificed. The abdominal cavity was exposed and the reproductive organs examined. Following removal of the uterus and ovaries the remains were subjected to macroscopic examination and the liver and thymus were weighed.
The uterus and ovaries of each animal were examined grossly. The number of corpora lutea per ovary was recorded. Ovaries of non-pregnant females were examined and then discarded. The uterine contents of each pregnant animal were exposed and the number and location of all implantations were recorded. The uterus of each female that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation. Blood samples were collected at the time of sacrifice and these were used for a range of serum chemistry determinations.
Each live fetus was weighed and its sex determined and examined for external anomalies. After gross examination the fetuses were equally distributed into two groups. One group was processed for an evaluation of visceral anomalies and the other group for skeletal anomalies.
Females in the postnatal groups with surviving offspring were sacrificed on post partum day 4 and the abdominal cavity was exposed for a gross examination of the reproductive organs. After examination, the uterus was discarded. All organs were examined macroscopically and the thymus and liver were weighed.

**Remark** : This study was also reported in the open literature by Feuston et al.(29)

**Result** : There were only few clinical observations that were considered to be treatment-related and these were confined to the high dose group (2000 mg/kg/day). The observations consisted of a red vaginal discharge, and one female in this group was pale in color. Slight skin irritation occurred in a few animals but it is not clear whether this was compound-related or self inflicted because the animals attempted to groom themselves, despite the fact that they had been fitted with collars. It is possible also that because of this some ingestion of test material may have occurred.
Animals in the 500 and 2000 mg/kg/day groups gained less weight than the controls over the gestation period (80% and 60% of controls respectively).

Food consumption for these two groups was also less than controls for the first 10 days of gestation but was similar to controls thereafter.
At necropsy, it was noted that there were more animals in the 2000 mg/kg/day group with a small thymus. Absolute and relative thymus weights were reduced in this group by approximately 50%. A reduction in absolute thymus weight was also recorded for the 500 mg/kg/day group, but the difference was not statistically significant.
Liver weights (absolute and relative) were increased in the 500 and 2000 mg/kg group, but only the relative organ weights were statistically significant (11 and 18% increases for the 500 and 2000mg groups respectively).
Serum chemistry differences were only observed in the 500 and 2000 mg/kg/day groups These changes were as follows:

| **Parameter** | **Dose group (mg/kg/day)** | |
|---|---|---|
| | **500** | **2000** |
| AST | | +27% |
| ALT | | +37% |
| Alkaline phosphatase | | +64% |
| Cholesterol | | +22% |
| Triglycerides | -42% | -62% |
| Total bilirubin | -35% | -49% |
| A/G ratio | | +15% |
| Phosphorus | | +20% |
| SDH | | +67% |

All other serum chemistry measurements were unaffected by treatment.

Reproductive evaluations
The only effect was a significant increase in mean number/% resorptions (6-fold) and a corresponding decrease in litter size (38%) in the 200 mg/kg/day group.

Fetal evaluations
Mean fetal body weights were reduced by approximately 13% in the 200 mg/kg/day group.
There was an increase in incomplete ossification of various skeletal structures which included nasal bones and vertebrae but no increases in visceral abnormalities. The data are shown below:
Date are shown as fetal incidence/litter incidence

| **Anomoly** | **Dose group (mg/kg/day)** | | | |
|---|---|---|---|---|
| | **0** | **125** | **500** | **2000** |
| Ossification variants | | | | |
| nasal bones | 1.0/8.3 | 15b/55 | 33b/82b | 96b/100b |
| Thoracic centra | 4/33 | 5.5/36 | 8.5/55 | 15a/78 |
| Caudal centra | 1/8.3 | 9.9a/36 | 3.7/36 | 15b/67b |
| Sternebrae <2 | 2/8.3 | 6.6/36 | 7.3/36 | 23b/67a |
| Visceral malformations | | | | |
| Right-sided esophagus | 0/0 | - | 0/0 | 4.1/22 |

a P<0.05
b P<0.01

In the prenatal group, the litter effects were;

Three females had no viable offspring, their litters were totally

resorbed

Two females had their entire litters die by postpartum day 3

There was a significant increase in the number of stillborn pups (7 compared to 2 for controls)

Liveborn pups weighed less than the controls (-8% and -17% on days 0 and 4 respectively).

**Conclusion** : Lost Hills Light crude was shown to be maternally toxic at 500 and 2000 mg/kg/day and developmentally toxic at 2000 mg/kg/day.

**Reliability** : (1) valid without restriction

(29) (68)

### 5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

**Type** : Study for sperm abnormality
**In vitro/in vivo** : In vivo
**Species** : mouse
**Sex** : male
**Strain** : other: B6C3F1/Hap hybrid
**Route of admin.** : i.p.
**Frequency of treatm.** : Once daily for 5 days
**Duration of test** : 40 days
**Doses** : 1.0 & 2.1 g/kg
**Control group** : yes
**Year** : 1982
**GLP** : no data
**Test substance** : Wilmington crude

**Method** : Groups of five 10-12 week old male mice were injected i.p. daily for 5 days with test samples in trioctanoin at doses of 1 and 2.1 g/kg. Control mice were injected with trioctanoin (5x0.25 ml) or BaP (5x0.05 or 1x0.07g). After 35 days, the animals were sacrificed, the cauda epididymes were removed, combined and minced in 0.9% NaCl or Tyrode's solution and the sperm were stained by adding eosin Y solution. The preparations were spread on slides and air-dried. The percentage of abnormal sperm was determined in 500 sperm from each animal. For control of bias in scoring, positive and negative control slides from a previous experiment were included at a ratio of 1 bias control slide to 5 new slides.

**Result** : The data from the study are given in the following table and show that treatment with Wilmington crud did not cause an increase in the percentage of abnormal sperm.

| Sample | Dose | No. survivors/ no. treated | Abnormal sperm (%±SD) |
|---|---|---|---|
| Trioctanoin | | | |
| | 5x0.25 ml | 10/10 | 4.5±1.7 |
| Wilmington crude | | | |
| | 5x2.1 g/kg | 5/5 | 6.1±2.1 |
| | 5x1.0 g/kg | 5/5 | 5.4±1.3 |
| BaP | 5x0.05 g/kg | 10/10 | 19.7±16.6 |

(43)

## 5.9 SPECIFIC INVESTIGATIONS

**Endpoint** : Mechanistic Studies
**Type** : Initiation/promotion assay
**Species** : mouse
**Sex** : male
**Strain** : Charles River (ChR-CD)
**Route of admin.** : Dermal
**No. of animals** : 30
**Vehicle** : undiluted
**Exposure period** : 180 day(s)
**Frequency of treatm.** : See method
**Doses** : See method
**Control group** : yes
**Year** : 1981
**GLP** : no data
**Test substance** : South Louisiana crude

**Method** : The authors summarized the protocol in the following table.

Animals: Charles River (ChR-CD) male mice Six weeks old
Toe clipped for identification
Backs shaved free of hair

Group size 30 mice per group

Housing 2 mice per cage

Diet Water and laboratory chow ad libitum

Observation Daily

Clipping of hair on the back Weekly, as needed

Treatments
Initiation: 50µl material as received
Promotion: 2 weeks after initiation treatment
2.5µg phorbolmyristate acetate (PMA)/0.1ml acetone
Three times a week for 180 days

Parameters measured
Body weight, recorded weekly
Time to development of first tumor
Tumors charted monthly
Mortality

**Result** :

| Sample | No. mice with tumors lasting >30 days | Av No. tumors/ tumor-bearing mouse | No. days to first mouse with tumor |
|---|---|---|---|
| Benzo[a]pyrene positive control | | | |
| | 26 | 6.0 | 48 |
| South Louisiana Crude | | | |
| | 5 | 1.4 | 71 |
| Toluene and PMA | | | |

| | | | |
|---|---|---|---|
| | 4 | 1.5 | 111 |
| PMA alone | | | |
| | 3 | 1.0 | 92 |

The authors concluded that the South Louisiana crude oil was not an initiator.

**Reliability** : (4) not assignable
The study was a preliminary screen and was not fully reported. The data are however are useful in assessing the tumor initiating activity of crude oil.
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